T113
YALE UNIVERSITY LIBRARY
3 9002 06679 0388
T113
+Y12
2555

A HISTOLOGICAL AND HISTOCHEMICAL STUDY OF TRANSPLANTED PITUITARY GLANDS IN MICE

THOMAS W. TILLACK

1963

MUDD
LIBRARY
Medical

YALE

MEDICAL LIBRARY


Digitized by the Internet Archive
in 2017 with funding from
Arcadia Fund

https://archive.org/details/histologicalhist00till

A HISTOLOGICAL AND HISTOCHEMICAL STUDY OF TRANSPLANTED PITUITARY GLANDS IN MICE

Thomas W. Tillack, A.B.
The University of Rochester, 1959

A Thesis Presented to the Faculty
of the Yale University School of Medicine
In Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

The Department of Anatomy
Yale University School of Medicine
April 1963

YALE MEDICAL
JUN 1963
LIBRARY
T113
Y12
2555

ACKNOWLEDGEMENTS

I wish to sincerely thank Dr. W. U. Gardner for his thoughtful advice and assistance in every phase of this project. Working with Dr. Gardner has been a stimulating experience, and his dedication to research, integrity, and interest in students will always be a source of inspiration. Thanks go also to Mrs. Mary Ann Pawlikowski for help of every sort during the four years in the laboratory, to Mr. Ling Chen for instruction in the techniques of histology, and to Dr. E. S. Crelin for instruction in the techniques of photomicrography.

This work was supported in part by three Josiah Macy Summer Fellowships.

TABLE OF CONTENTS

INTRODUCTION

The role of the anterior lobe of the pituitary gland in the control of the other endocrine glands and the reproductive system has come under intensive investigation in recent years. Especially since the function of the posterior lobe of the pituitary and its direct connection to the hypothalamus has been elucidated, the relation of the anterior lobe to the hypothalamus and the complex control system which exists between hypothalamus and anterior lobe and their response to feedback hormones from other endocrine glands has been of increasing interest. The function of the hypothalamic-pituitary portal system in the transport of blood and hormonal messages from the hypothalamus to the anterior lobe of the pituitary has also been under investigation.

Several methods have been used to investigate the basic function of the anterior pituitary gland. Anterior pituitary hormones obtained from chemical separations of active principles in the whole gland and similar substances derived from blood and urine have been employed to study their actions on gonads and other accessory organs of reproduction. The techniques of hypophysectomy and transplantation of the pituitary gland have been used to study function of the gland. However, basic to all these methods is an elucidation of the cytology of the pituitary gland and an histochemical identification of the actual hormones in the gland. Also, the

effects of gonadal hormones and of different endocrine states in the animal and their effects on pituitary cytology and histochemistry have been studied.

Much of the classical work on the cytology of the anterior pituitary gland was done during the 1930's. Severinghaus (53) has reviewed anterior pituitary cytologic knowledge prior to 1939. He described three types of pituitary cells: chromophobes, cells with a cytoplasm that was devoid of granules that could be specifically stained; acidophiles, those cells that contained granules stained with plasma dyes; and basophiles, those cells whose cytoplasmic granules stained with basic dyes. These three cell types were present in pituitary glands of all species examined and were considered to be the only established cell types of the normal anterior hypophysis. Three specialized cells are found after castration, after thyroidectomy, and during pregnancy. Castration frequently results in a marked increase of basophiles. Certain of the enlarged basophiles become vacuolated with a colloid-like substance that crowded the nucleus and Golgi apparatus to the cell periphery. This is the "castration cell" or "signet ring cell." A similar cell, called a "thyroidectomy cell," is produced after removal of the thyroid gland. These cellular changes were thought to represent increased hormone storage. Pregnancy changes in the anterior lobe indicate excess activity, both of production and release of hormone, with a depletion of stored product. Many cells are depleted of both their acidophilic and basophilic granules and have a hyper-

trophied Golgi apparatus and abundant mitochondria. About the time of parturition, at least in the guinea pig, the number of acidophiles increases and persists after the birth of young.

The injection of estrogens prevented the changes incident to castration. In addition, estrogens depleted the staining reaction of the granular cells if given in larger than physiologic doses. The marked degranulation of the basophiles and acidophiles in the hypophyses of estrogen-treated animals is considered to be cytological confirmation of secretion release, as is also the hypertrophy of the mitochondria.

It soon became apparent that two different types of basophiles existed in the anterior lobe of the pituitary gland. Purves and Griesbach (47, 48) were among the first to elaborate clearly these two cell types using the Periodic Acid Schiff reagent (PAS) to stain for glycoprotein, as recommended by Catchpole (9). PAS had been shown to stain glycoprotein, and basophiles which contain glycoprotein stain blue with the aniline blue in the Mallory technique. Purves and Griesbach distinguished two types of glycoprotein-containing cells: One type was an oval or rounded cell that stained intensely by PAS and that was localized to the lower surface of the anterior pituitary and to the upper surface adjacent to the pars intermedia. Cells of this type became degranulated in animals given estrogen and became the castration cells in gonadal deficiency. The PAS reaction in these cells was correlated with the known content of gonadotrophic hormone of the gland under experimental conditions. Purves and Griesbach therefore concluded that cells of this type are the site of production of gonadotrophic hormone and the name

"gonadotroph" was suggested for them. The second type of cell that contained glycoprotein was polyhedral and these cells occupied the central region of the anterior lobe. Cells of this type were degranulated in animals given thyroxine, and in thyroxine deficient animals they became thyroidectomy cells. The intensity of the PAS reaction in these cells was correlated with the content of thyrotrophic hormone in the gland. These cells, for which the name "thyrotrophs" was suggested, were assumed to be responsible for the production of the thyrotrophic hormone.

Other investigators have reported similar observations. Halmi (19, 20, 21, 22) noted two different types of basophiles in pituitary glands stained with aldehyde fuchsin combined with a modified azan method. One cell type, designated beta cells, stained with the aldehyde fuchsin, whereas another type, delta cells, stained green in this method. Later, the "beta" cells were equated with the "thyrotrophic" cells of Purves and Griesbach and the "delta" cells with the "gonadotrophs." The aldehyde fuchsin material of the beta cells was identical with the PAS staining granules of the thyrotrophs. Aldehyde fuchsin, therefore, was concluded to be a specific stain for thyroid stimulating hormone (TSH). Granules of the delta cells also gave a strong reaction with PAS and were therefore concluded to contain follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Pearse (41, 42, 43, 44) used a trichrome PAS stain (containing Orange G as an acidophilic stain) to investigate

the localigation of anterior pituitary hormones. He states that this stain distinguished between mucoid cells containing "beta" granules and those containing "gamma" granules, which he later equated with the "beta" cells and "thyrotrophs" of Halmi and Purves and Griesbach, and with the "delta" cells and "gonadotrophs", respectively. Paget and Eccleston (39, 40) describe an aldehyde thionine stain which has properties similar to aldehyde fuchsin and which selectively stains thyrotrophs, but which is more stable and consistant than aldehyde fuchsin. Adams and Swettenham (1) have identified these two types of basophile cells histochemically in the normal human anterior pituitary gland.

Several authors have distinguished two types of gonadotrophs. Purves and Griesbach (49) claimed it was possible to divide the gonadotrophic basophiles on morphological grounds into two subtypes, one secreting FSH and the other LH. The FSH-secreting cells are oval, PAS-positive, aldehyde-fuchsin negative peripheral gonadotrophs that are concentrated in two lateral ridges on the superoanterior surface of the anterior lobe of the pituitary ("sex zone"); the LH secreting cells are PAS positive, aldehyde-fuchsin negative central gonadotrophs which are widely dispersed. A comparable distribution of the gonadotrophs is also reported by several other investigators (5, 24, 27, 50, 55, 60).

Barrnett, Ladman, AcAllaster and Siperstein (4, 5) also distinguished between FSH and LH secreting gonadotrophs. Glycoprotein hormones (FSH, LH, TSH) were localized in the

cells of the anterior pituitary gland of the rat by combined use of differential protein solubilities, staining procedures, and bio-assays. Rats' pituitary glands immersed in 2.5% trichloracetic acid (TCA) had their FSH and TSH extracted completely, but retained LH as determined by bio-assays. Those cells that contained PAS-positive material were stained less intensely, and aldehyde-fuchsin staining was lacking. The FSH and TSH were dissolved and the residual PAS reaction was attributable to LH. All three glycoprotein hormones were localized in PAS-positive cells, and TSH was contained in aldehyde-fuchsin positive cells. LH was found in PAS-positive cells scattered throughout the gland and was not regionally confined to the "basophiles" of the "central zone." Furthermore, LH was frequently associated with FSH, especially in PAS-positive cells of the "sex zone" of normal rats, whereas this association was less apparent in the "central zone" cells.

THE MORPHOLOGY, HORMONE CONTENT AND FUNCTION OF ACIDOPHILES

The acidophiles of the anterior pituitary gland are presumed to be the sources of somatotrophic hormone (STH), luteotrophic hormone (LTH), and possibly adrenocorticotrophic hormone (ACTH). At least two types of acidophiles are distinguishable on morphological grounds by using staining techniques in some species, but this distinction is unclear and is the source of confusion in attempting to equate cell types with specific hormone production. In 1938 Dawson

and Friedgood (11, 14) described the differentiation of two types of pituitary acidophiles in the cat and rabbit using a modification of Heidenhain's "azan" stain. The typical acidophile stained with Orange G, and a special type of acidophile stained with azocarmine. Later Dawson (12) found that the number of azocarmine-staining (carminophile) cells increased greatly shortly after coitus and during the latter part of pregnancy in the pituitary of the cat. These cells degranulated during lactation, and it was suggested that the carminophile cells were associated with LTH production. Dawson (13) also demonstrated these two types of acidophiles in the pituitary gland of rats. Orange G-azocarmine discerned between cells in only certain species (not including the mouse), and in those species in which two types of acidophile can be distinguished, one type may be expected to secrete STH and the other LTH (46). One of the two specific cell types is reactive in relation to the reproductive cycle and shows marked fluctuations in activity which can be correlated with the secretion of LTH at times of expected high LTH secretion; the other type is relatively stable in relation to the reproductive cycle, and is assumed to secrete STH. Mammals in which the carminophile cells are active during pregnancy and lactation are the cat, rabbit, monkey and bat. Mammals in which the Orange G cells are active and in which the carminophile cells are stable are the human and rat. Sanders and Rennels (52) found that LTH-secreting pituitary

autografts placed under the renal capsule contained predominently cells that stained with Orange G and therefore concluded that the Orange G-staining cell of the pituitary gland produces LTH.

Barrnett and Ladman (31) described the staining of acidophiles of the anterior pituitary of the rat with the Barrnett-Seligman (2, 3) method for histochemical demonstration of sulfhydryl (SH) and disulfide (S-S) groups of protein. The histochemical method for these groups was specific and the sites of the histochemical reaction were at the same loci as the reactive groups in the protein. Basophiles and chromophobes contained little or no SH or S-S groups. Since most of the protein hormones of the adenohypophysis contain disulfides and the acidophiles stain strongly for these groups, the investigation was undertaken to determine histochemically under what experimental conditions alteration of the amount of protein-bound SH and S-S groups occur. The authors concluded that the alterations in the staining of the acidophiles following acute and chronic stress or adrenalectomy indicated that the production of ACTH is connected with this cell type.

The localization of LTH in the acidophiles of the rat pituitary gland and the suggestion that LTH may be produced by only certain of the acidophiles was reported by Barrnett, Roth and Salzer (6, 8). When rat pituitary glands, taken from normal animals, were immersed in 0.5% trichloracetic acid (TCA), only LTH was precipitated; the remainder of the anterior pituitary hormones were dissolved and extracted

as proven by bio-assays and *in vitro* tests. When such extracted glands, containing a normal amount of only LTH, are fixed in histological fixatives, sectioned and stained by histochemical methods that specifically reveal the sites of either sulfhydryls (31) and disulfides or carboxyl groups (7), only the granular cytoplasm of *some* of the acidophiles were reactive. Basophiles, chromophobes and the remainder of the acidophiles were unreactive. In addition, the number of cells in glands of pregnant and lactating rats that reacted histochemically after extraction in 0.5% TCA varied directly with the amount of hormone present; thus in 3 day post-partum rats where LTH activity and secretion is considered to be maximal, almost all of the acidophiles extracted in 0.5% TCA reacted, substantially more than in the control rat pituitary. Thus Barrnett et al. conclude that a specific class of acidophiles is probably responsible for LTH production.

Further proof that the acidophiles are the source of LTH was offered by Hymer, McShan, and Christiansen (28). Electron microscopic study of the anterior pituitary glands from lactating and estrogen-treated rats revealed that the acidophiles developed an extensive reticulum indicating that they probably elaborate LTH. Rennels (51) studied pituitary transplants in the rat with the electron microscope and concluded that a specific type of acidophile in the pituitary graft produces LTH.

Evidence that the acidophiles are associated with the production of STH is derived from several sources. Cushing and Davidoff (10) in 1927 first deduced that acidophiles produce growth hormone from observations of acidophile cell adenomas in the pituitaries of patients showing the symptoms of acromegaly or gigantism. Smith and MacDowell (58) in 1930 demonstrated the absence of acidophiles in the anterior lobe of the pituitary of the dwarf mouse, indicating that a growth-regulating hormone is produced in these cells. Elftman and Wegelius (15) confirmed a deficiency in typical acidophiles in the dwarf mouse and also noted the almost complete absence of thyrotrophs. Leznoff, Fishman, et al. (32) used the fluorescent antibody technique and demonstrated that labelled rabbit antibodies to Raben human growth hormone localized specifically in acidophilic cells of the human anterior pituitary gland and in acidophile adenomata from pituitaries of patients with acromegaly.

One additional stain for the acidophiles of the anterior pituitary gland was proposed by Shanklin, Nassar and Issidorides (54) in 1959. Luxol fast blue was demonstrated to stain acidophiles of the anterior pituitary selectively. Luxol fast blue had the advantage of retaining its intensity when combined with the periodic acid-Schiff procedure, and it imparted an intense blue color to the acidophiles which contrasted with the red or magenta staining of the PAS-positive cells.

TRANSPLANTATION OF THE PITUITARY GLAND:

Transplantation of the intact pituitary gland has provided a method of investigating the interaction between pituitary and hypothalamus. In 1935 Haterius, Schweizer, and Charipper (23) reported that rabbit and guinea pig pituitary glands transplanted to the anterior eye chamber persisted for at least 4 months. The grafts retained their histologic identity and underwent some degree of hyperplasia, but no evidence of established vascular connections to the eye was found. In 1939 Loeb and Kirtz (33) demonstrated that multiple transplants of the anterior lobe of the pituitary from mice of a closely inbred strain into the subcutaneous tissue of their brothers and sisters survived for 7 to 10 months, induced intensified growth and secretion processes in the mammary gland tissue of the host, and accelerated the carcinomatous transformation of the stimulated mammary gland tissue in many cases. They associated the appearance of the increased number of mammary tumors at an earlier age and the increased activity of the mammary glands with a direct action of the pituitary graft in addition to an indirect action through the ovary, many of which contained abundant luteal tissue. These mice, it has been subsequently shown, had the mammary tumor agent.

Everett in 1954 (16) reported that functional corpora lutea were consistently obtained in rats bearing hypophyseal autografts without any stimulus other than transplantation itself. In 1956 (17) he transplanted the anterior pituitary

gland of female rats autologously into the renal capsule on the day after ovulation and tested for corpus luteum function, and indirectly for LTH secretion, by measuring vaginal mucification following administration of an excess of estrogen. He thus demonstrated that removal of the anterior pituitary from its normal site favors LTH secretion and allows corpora lutea to remain functional at least 4 or 5 times the duration of pregnancy, indicating that the functional life of rat corpora lutea is not self-limiting, as often is postulated. Transplantation of the pituitary gland removed the luteolytic mechanisms which operate in the intact rat. Nikitovitch-Winer and Everett (35, 36, 37) have proved functional restitution of pituitary grafts in rats retransplanted from the kidney capsule to the median eminence. In 14 rats the autograft on the kidney capsule was re-transplanted after 3 to 4 weeks close to the median eminence. Estrous cycles returned spontaneously in 13 animals and 7 became pregnant. Ovaries, follicles, and corpora lutea were histologically normal. In control rats in which the graft was re-transplanted from kidney to a site under the temporal lobe and in the rats in which the graft remained on the kidney there was no sign of return of FSH or LH secretion. Studies on TSH and ACTH secretion indicate that a residual secretion of these hormones can proceed without a close linkage to the hypothalamus, ie., even when the pituitary is transplanted under the kidney capsule, but these authors found that even greater secretion of TSH and ACTH was evident in animals with pituitaries

re-transplanted under the median eminence. From these studies they conclude that extreme deficiencies in pituitary trophic hormones, except for LTH, result when the anterior lobe is removed to sites distant from the hypothalamus, and that these deficiencies are caused by the loss of hypothalamic influences, which are probably mediated by the hypophysial portal vessels which constitute the principal link between median eminence and the anterior lobe of the pituitary. The secretion of LTH, on the other hand, appears to be enhanced by removal of the anterior lobe from the hypothalamus; thus in the normal state, the secretion of LTH must be held in check by the hypothalamus.

Smith (57) extended and confirmed these observations in an experiment in which he homotransplanted pituitaries in male rats which had been hypophysectomized for 60 to 150 days into the region of the median eminence. After 2½ months of transplantation, he noted that all transplants had become functional, as indicated by gains in body weights, activation of the thyroids, structural repair of the adrenals and the reproductive organs, and fertility.

Growth hormone is apparently produced to some degree in transplanted pituitary glands. Hertz (25) reported that somatic growth at 2/3 of the normal rate was maintained in the hypophysectomized rat bearing four pituitary transplants.

The stimulation of mammary gland growth and lactation in mice bearing transplanted pituitary glands provided evidence of their production of greatly increased amounts of LTH. Muhlbock and Boot (34) showed that the subcutaneous

transplantation of anterior pituitary glands in mice was associated with a continuous LTH release by the grafts and that mammary tumors were induced in female animals of six inbred strains, all without the mammary tumor agent, by subcutaneous isografts of 20 to 200 hypophyses. The luteotrophic effect of the grafts was confirmed by checking daily vaginal smears and observing the function of corpora lutea indicated by repeated pseudopregnancies. Thus they conclude that the mammary glands were under continuous stimulation by large amounts of LTH and progesterone, and normal or decreased amounts of estrogen, resulting in proliferation and tumor development. In addition, they found that the grafted pituitaries in one hybrid strain of mice developed into tumors.

Gardner (18) found that pituitary tumors developed in pituitary glands transplanted subcutaneously into female mice of two hybrid groups and of two inbred strains. The incidence of mice with tumors ranged up to 100 per cent, but the tumors appeared only in mice that survived 500 days or more. Mice bearing the pituitary grafts or tumors showed well-developed mammary glands and lactation, but usually the uteri or vaginal mucosae were atrophic unless the ovaries were functioning. Vaginal smears taken in younger mice bearing three or four pituitary transplants had prolonged anestrous periods and only brief periods of vaginal cornification, and when one or two pituitary grafts were implanted, the mice tended to have pseudopregnancy-like cycles. The pituitary transplant tumors were composed of nongranular cells, referred to as chromophobic

cells. Most of the tumor cells were larger than those of the usual transplant and somewhat larger than those in the normal pituitary, although some tumors were composed of small cells, similar to those found in nontumorous pituitary grafts. Gardner thus showed that tumors arising in the pituitary grafts continued to produce LTH effects as judged by the mammary and ovarian responses, and postulated that long-sustained exposure of the graft to the LTH might provoke pituitary as well as mammary tumorigenesis.

Several investigators have studied the histological and histochemical characteristics of transplanted pituitary glands in an effort to ascertain which of the pituitary cells persist and to determine hormone content of the graft cells. Wolfe, Kirtz, and Loeb in 1940 (62), using a hematoxylin and eosin and a Mallory triple stain, studied the histology of subcutaneous pituitary transplants in mice. Transplants were found alive after periods of from 7 to 10 months after transplantation. The persisting transplants were surrounded by a fibrous capsule from which strands of connective tissue grew into the graft. The surviving anterior lobe tissue was made up chiefly of chromophobes, but in a majority of the transplants a few eosinophiles were seen. Basophiles were not observed. In some grafts intermediate lobe tissue was identified. These authors made cell counts in a few of the anterior pituitary glands of the host mice which suggested that basophiles were more abundant in the glands of ovariectomized host mice than in glands of non-ovariectomized animals, and these were

generally degranulated. Castration cells were not observed.

Nikitovitch-Winer and Everett (38) studied the histologic changes of rats' pituitary grafts on the kidney and upon re-transplantation under the diencephalon, using the PAS and aldehyde-fuchsin (AF) techniques. Time intervals from transplantation to autopsy ranged from 1 day to 6 months. They found that transplants to the kidney are severely damaged by the transfer, the functional parenchyma of the graft being derived from a thin shell of healthy tissue surrounding the massive central infarct. Reorganization of the graft was essentially complete after one week. Normal cytologic differentiation was promptly lost, with large gonadotrophs (PAS+, AF-) being absent, but smaller ones being found in significant numbers for 2 to 3 weeks, after which they were rarely recognized. Thyrotrophs (PAS+, AF+) were prominent during the first week, but older grafts contained a few angular basophiles, sometimes vacuolated. Small chromophobes were abundant. The changes of the acidophile population were not evaluated. Grafts re-transplanted under the median eminence and examined at autopsy several months later had recovered much of their normal cytologic characteristics. The return of large gonadotrophs was the main feature, with "castration cells" dominating in animals whose ovaries remained inactive. Large thyrotrophs were common.

Siperstein and Greer (56) used the PAS and Mallory triple stain to study the histocytology of the mouse pituitary transplanted to the anterior eye chamber. Pituitaries of

newborn mice were implanted in the anterior eye chambers of normal female mice of the same strain and the resulting viable transplants were taken for cytologic study at intervals of from 1 day to 14 months. They found that during the first 8 days of transplantation, the pituitaries were in stages of degeneration or necrosis. Anterior-lobe tissue grew well subsequent to the first week after transplantation. The intensity of staining of anterior-lobe cells was much less than that of the normal gland; acidophiles appeared less frequently with time until they were very rare by the 51st day, while just a few basophiles were discernible in a few specimens in the 15 to 36 day groups. The chromophobes were larger than those in normal pituitaries and had cytologic manifestations of activity. The intermediate lobe, after the first week, exhibited considerable capacity for growth, with numerous mitoses, PAS staining, and negative Golgi images. The neural lobe could be seen in implants up to 8 days, but its presence after this could not be ascertained.

A similar study of histological alterations in the rat pituitary transplanted to the eye was reported by Kovacs (30). He found that the center of the grafts became necrotic and was surrounded by a peripheral zone of live cells. He did not find evidence of regeneration or atrophy of this live peripheral zone and no mitoses were observed there. After 6 weeks only very rare shrunken basophile cells remained, but a few acidophile cells could be identified. The chromophobes became completely degranulated during the first week or two.

Knigge (29) transplanted neonatal rat pituitary glands to the anterior eye chamber of hypophysectomized and intact male hosts, and studied the transplants histologically after from 2 to 45 days using an aldehyde fuchsin-Masson stain. He noted the usual central necrosis with a hyperplasia of the surviving pituitary tissue at 13 days. At 30-45 days after transplantation, the majority of graft cells were small chromophobes, and significant amounts of acidophilic granulation were not seen in any cells, regardless of age of the graft. Basophiles ranging in size from 9-15 micra in diameter were recognized, but no AF positive basophiles were observed. Cells of the intermediate lobe exhibited extensive mitotic activity and hyperplasia when transplanted, and neural elements were replaced by dense connective tissue.

MATERIALS AND METHODS

ANIMALS:

Mice from a hybrid group developed by Dr. W. U. Gardner and designated PC (C_3H X BC) were used in this study. All mice were raised in Dr. Gardner's laboratory and maintained by brother-to-sister mating. Only adult male and female mice were used, ranging in age from 108 to 234 days, and in weight from 19 grams to 33 grams, the majority weighing from 22 to 28 grams.

DIET AND HOUSING:

Mice were housed in steel cages on sawdust, five to six animals per cage. Constant temperature and humidity conditions were maintained. Mice were fed a diet of Purina Laboratory chow and water ad libitum. Following hypophysectomy mice were, in addition, offered 1% saline and 2% glucose solution.

HORMONES:

Estradiol Benzoate: Estradiol benzoate was dissolved in sesame oil, so that 25 ug. would be contained in 0.05 ml. of sesame oil. After diluting the hormone solution, the mixture was allowed to sit for 24 hours to allow thorough diffusion of the hormone in the oil. Mice receiving estrogen were injected twice weekly with 25 ug. portions of estradiol benzoate into the subcutaneous tissue of the back. (See Footnote 1)

OPERATIVE PROCEDURES:

Hypophysectomy: (Gardner modification of the Thomas Method (59))

The mouse received subcutaneously 0.1 gm/Kg. of body

(1: Estradiol Benzoate, No. 14855. Organon, Inc., Orange, N.J.)

with a forceps. The testicular artery, epidydimus, etc., were clamped proximal to the testis, and the testis was freed by cauterizing along the border of the clamp. The remaining tissue was replaced into the peritoneal cavity, and the wound was closed with silk sutures.

Female mice: Following induction of ether anesthesia the back was shaved and a longitudinal medial incision was made into the retroperitoneal space. The ovary, oviduct, and upper part of the uterine cornu were exteriorized into the wound with a forceps. The ovary and oviduct were freed from the uterus by cauterizing along the border of a clamp placed proximal to the ovary and oviduct. The remaining tissue was replaced, and the wound closed with silk sutures.

METHOD OF PITUITARY GLAND TRANSPLANTATION:

Host male and female adult mice that were to receive one, two, or rarely three, whole pituitary glands subcutaneously were divided into six groups:

Group 1: Normal male and female adult mice.

Group 2: Castrate male and female adult mice.

Group 3: Hypophysectomized male and female adult mice.

Group 4: Hypophysectomized and castrated male and female adult mice.

Group 5: Male and female adult mice receiving twice weekly injections of estrogen.

Group 6: Normal male and female adult mice that received no pituitary transplant and that served as control animals.

Castrations of all mice were performed on the day prior to transplantation. Hypophysectomy was done two or three days prior to transplantation. Estrogen injections were begun on the day of transplantation.

The pituitary glands used for transplantation were obtained from donor mice of the same hybrid group as the host mice. In all cases female donor pituitaries were placed in female host mice, and male donor pituitaries were placed in male host mice. One, two, or rarely three whole pituitary glands, both anterior and posterior lobes, were transplanted subcutaneously along the lateral body walls of host animals. When one pituitary gland was transplanted, it was placed on the right side; when two were transplanted, one was placed on the right side and one on the left.

The donor mice were killed with gas, and the pituitary glands were removed and transplanted usually within two minutes or less after death of the donor. After removal from the donor, the pituitary glands were placed on a sterile saline-dampened filter paper in a Petri dish and then taken up in a 13- or 16-guage hypodermic needle fitted with a plunger. The glands were then implanted in the subcutaneous space of the lateral body wall. An attempt was made to keep the pituitary glands as intact as possible during removal and implantation.

VAGINAL SMEARS:

Smears of the exfoliated vaginal cells of all of the female control and host mice were made for periods of from 3 to 8 days before sacrifice of the animals. These smears

were stained in hematoxylin and eosin, and served to indicate the functional activity of the ovaries and indirectly of the pituitary grafts.

METHOD OF AUTOPSY:

At autopsy, the mice were anesthetized deeply with ether and the pituitary gland was exposed by removing the calvarium and reflecting the brain. A wedge of bone from the sella was removed with attached pituitary and dura, and this was immediately placed in fixative. After fixing for 15 to 30 minutes, the pituitary was dissected from the bone and dura with minimal trauma to the pituitary gland. This method of killing the mice was adopted to avoid exciting the animal and thereby possibly depleting the pituitary of hormones, particularly ACTH.

In hypophysectomized mice, the sella turcica was inspected for any evidence of pituitary tissue.

Next the skin was removed by making a dorsal incision from the vertex of the skull to the root of the tail. The skin, containing the subcutaneous tela and mammary glands, was pulled free from the carcass and in the ventral areas around the limbs blunt dissection was used to bring away the fatty tissue and mammary glands with the skin. The pituitary grafts were immediately dissected free from the subcutaneous tissue and placed in fixative. The skin containing the subcutaneous tela and mammary glands was fixed in Bouin's fluid, and after 24 hours, the mammary glands were dissected free, stained with hemalum, and mounted using Damar in xylol.

In female mice, the ovaries, uterus, adrenals, vagina,

submandibular glands, and sometimes the kidneys were dissected free of adjacent tissue and weighed. In male mice, the adrenals, submandibular glands, testes, seminal vesicles and prostate, and sometimes the kidneys were dissected and weighed. The interpubic ligament was measured in all animals with a Vernier caliper.

METHODS OF FIXATION, SECTIONING, AND STAINING OF PITUITARY GLANDS AND GRAFTS:

Three male and three female mice in each of the six groups of host animals (see page 21) were autopsied at varying intervals of time after a pituitary graft was transplanted subcutaneously: at 4 days, 7 days, 14 days, 1 month, and 2 months after transplantation. A few animals receiving transplants were autopsied at 12 hours, 1 day, and 2 days, and the grafts recovered from the subcutaneous space.

The pituitary gland and subcutaneous pituitary grafts of one male and one female mouse in each of the six groups were placed in sublimate formol fixative for four hours (45). This fixative consists of 9 parts saturated aqueous solution of mercuric chloride and 1 part formaldehyde. The glands and grafts were then washed, dehydrated, and embedded in paraffin in the usual manner. All six anterior pituitary hormones are precipitated by this method (8).

The pituitary gland and grafts of one male and one female mouse in each of the six groups were immersed in 0.5% trichloracetic acid (TCA) for 24 hours and then were fixed in sublimate-formol for four hours, dehydrated, and embedded. According

to Barrnett, Roth, and Salzer (8), rat pituitary glands immersed in 0.5% TCA retain only luteotrophic hormone in the acidophiles, the remainder of the anterior pituitary hormones being dissolved and extracted. This is thus a specific histochemical method for the demonstration of the sites of luteotrophic hormone in the rat pituitary.

The pituitary gland and grafts of one male and one female mouse in each of the six groups were immersed in 2.5% TCA for 24 hours and then were fixed in sublimate-formol for four hours, dehydrated, and embedded. Barrnett, Ladman, McAllaster and Siperstein (5) showed that this concentration of TCA extracted FSH and TSH completely from the basophiles of the rat pituitary, but did not remove luteinizing hormone. Thus, this method provides a histochemical method for the identification of LH.

Strips of adjacent sections of embedded pituitary were cut in the frontal plane at several different levels throughout the gland. Sections were cut at 4 micra in thickness. Representative sections at the different levels were then placed on glass slides. The pituitary grafts were cut at several different levels throughout the transplant, and representative sections at different levels were placed on slides.

One set of sections from each pituitary gland and graft was stained with a routine hematoxylin and eosin stain. The other set of sections from each pituitary gland and graft was stained in the author's modification of the Periodic Acid

Schiff, Luxol Fast Blue, Harris' Hematoxylin stain proposed by Shanklin *et al.* (54). This method was found to give the most satisfactory staining of the acidophiles in both the pituitary glands and the transplants, the Luxol Fast Blue staining acidophiles intense blue and remaining stable throughout the staining procedure.

TERMINOLOGY:

Throughout this paper, the term "basophile" will refer to a cell which is PAS positive because it contains glycoprotein. Purves and Griesbach (47) divided the basophiles, which they considered to have gonadotrophic function, into two types on the basis of their regional localization in the anterior lobe of the pituitary. One type occurred in the "sex zone," an area found in the anteromedial portion of the anterior lobe adjacent to the intermediate lobe. These basophiles were oval or rounded cells which were PAS positive, and were also called the "peripheral" cells or "gonadotrophs." The second type of PAS positive cell was found in the "central zone" of the anterior lobe, the mid-region of the lateral parts of the anterior lobe. These cells are polyhedral in shape and were referred to as "central cells" or "thyrotrophs."

The term "acidophile" refers to a cell that is Luxol Fast Blue positive, and which gives a strong histochemical reaction for protein-bound SH and S-S groups (31).

The term "chromophobe" refers to a cell that is not stained by either PAS or Luxol Fast Blue.

METHOD OF EVALUATION OF DATA:

Evaluation of pituitary transplants: At autopsy, the pituitary grafts were observed grossly, noting the presence or absence of the graft, state of intactness of the graft, blood supply of the graft, and the effect of the graft on the surrounding mammary tissue. Enlargement or tumors of the graft were searched for. Microscopically, the pituitary grafts were examined carefully, and the following items were noted: (1) The general cell population of the grafts was quantitated, noting presence of basophiles, acidophiles, and chromophobes and the relative staining intensity of these cell types as an indication of amount of hormone content. This was done for grafts that had been in place subcutaneously for from 12 hours to 2 months; (2) The inflammatory response, amount of necrosis and vascular response within the transplanted gland at varying intervals of time were quantitated; (3) Presence of mitotic figures in anterior lobe cells was noted; (4) The presence, size, and cell types and staining intensity of intermediate lobe tissue were recorded; (5) Other general cellular characteristics of the transplant were noted; (6) The sizes of representative basophiles and acidophiles were measured in the pituitary transplants at the intervals from 4 days to 2 months. This was accomplished by measuring the widest diameter of these cells with an ocular micrometer. The measurements of the cells of transplanted glands were compared with those of acidophiles and basophiles of the normal pituitary gland; (7) The microscopic characteristics of the grafts were

compared with respect to the 5 groups of animals which bore transplants (see page 21). Thus grafts in normal animals were compared to grafts that had been placed in castrated mice, hypophysectomized mice, and estrogen-injected mice; (8) The microscopic characteristics of the pituitary grafts were compared with respect to the three different methods of fixation used in this experiment (see page 24). Thus grafts that were fixed in sublimate-formol were compared with grafts extracted in 0.5% TCA and in 2.5% TCA prior to fixation with sublimate-formol.

Evaluation of pituitary glands of host animals: The pituitary glands of control mice and mice bearing pituitary transplants were examined microscopically, and the following items were recorded: (1) The percent of basophilic, acidophilic, and chromophobic cells were determined by cell count. Counts were done by inserting a mold counting lens in one eyepiece of a binocular microscope. This lens has three parallel lines running vertically and three parallel lines running horizontally, and the intersections of these lines form nine points. Each of these points rested on a different pituitary cell. One hundred pituitary cells were counted in each pituitary gland, and the differential count was recorded on a counter. The cells were counted under oil immersion to be certain of the identity of the pituitary cells. Thus 11 different fields were counted across the inferior border of each frontal section of pituitary giving a total of 100 cells counted for each pituitary gland; (2) The relative intensity of the basophiles and

acidophiles of the pituitary glands were noted as an indication of amount of hormone present; (3) The microscopic characteristics of the pituitary glands were compared with respect to the 6 groups of animals which bore pituitary transplants (see page 21). Thus pituitary glands in normal mice without transplants were compared with glands in mice bearing transplants, and with glands in castrate and estrogen-injected mice bearing transplants; (4) The microscopic characteristics of the pituitary glands were compared with respect to the three different methods of fixation used in this experiment (see page 24). In particular, the percentage and intensity of acidophiles of normal pituitaries fixed in sublimate-formol were compared with the acidophiles of pituitaries which were stained expressly for LTH, ie., were extracted in 0.5% TCA.

Evaluation of the effects of pituitary transplants on genital organs and other endocrine glands: The endocrine glands and genital organs of host mice were weighed at autopsy, and these weights were averaged for each group of mice and were plotted against the time in days for which the transplants were present. This provided information on the "trophic" effects of the pituitary graft, and provided a means of evaluating the function of the graft in castrate and hypophysectomized mice. Microscopic sections of representative ovaries were prepared to evaluate the state of follicular development and corpora lutea formation.

Evaluation of the effects of pituitary transplants on mammary glands: The second and third pairs of mammary glands

from host female mice and some male mice were mounted in Damar on glass slides. The development of the mammary glands was evaluated by microscopically evaluating the proliferation of the alveoli. The scale used for rating assigns the gland a value from 0 to 4; 0 indicates extreme atrophy and 4 indicates extreme alveolar proliferation with full lactation. If local proliferation around the site of implant of the graft was in excess of the development of the mammary glands at a distance from the graft, this was noted. Photographs of the mammary glands were obtained by placing the slide on the Omega Enlarger and making prints directly--thus a negative image of the mammary gland was obtained.

RESULTS

PERSISTENCE OF PITUITARY GRAFTS:

Most of the pituitary grafts to the subcutaneous tissues of male and female mice were found at autopsy (see Table 1). A total of 129 pituitary glands were transplanted subcutaneously into 87 male and female mice, and 109 of these grafts were located at autopsy, giving an 84% recovery rate. Most host animals received one pituitary transplant in the right lateral body wall, some received one in the right and one in the left body wall, and rarely, three pituitary glands were transplanted into one animal, two on the right and one on the left. Since pituitary glands of male donors were implanted in male hosts and of female donors in female hosts, no sex differences existed in histocompatability. All mice were of the PC hybrid group, and the members of this group showed a high degree of histocompatability. All mice were from 108 to 234 days old, that is, young adult mice, so no differences in the incidence of successful transplants as related to the age of the host or donor mice were observed.

Most of the recovered pituitary transplants were intact, that is, showed a shape and organization of the tissues similar to the intact gland. Some had become fragmented into two or three pieces during the transplantation procedure, but the fragments were usually in close proximity to each other. The grafts usually adhered to the subcutaneous tissue at the lateral body wall in the region of the second and third mammary

TABLE 1

NUMBER OF PITUITARY GRAFTS RECOVERED FROM INTACT HOSTS

No. Days Grafts In Place	Number Animals	Total No. Grafts Implanted	Total No. Grafts Recovered
4 days	3 male	3	3
	3 female	6	4
7 days	3 male	6	6
	3 female	6	6
14 days	3 male	6	5
	3 female	6	5
30 days	3 male	6	5
	3 female	6	6
60 days	3 male	6	3
	3 female	3	2
		54	45

83% Recovery of Grafts

glands, and were often found within the substance of the mammary gland itself. Grafts were found in castrate, hypophysectomized, and estrogen-injected mice bearing transplants in as high a percentage as in normal mice bearing transplants. Grafts usually had a good blood supply and appeared as small fleshy, pink masses within a capsule of connective tissue. This was true in grafts removed at 4 days and in grafts remaining in place for 2 months.

Enlargements or tumors were not observed grossly in the transplants at autopsy. The grafts usually ranged in size from approximately half the normal pituitary size to the size of the intact pituitary gland. Often the general topography of the pituitary gland was retained grossly in the graft, the pink lateral lobes of the anterior lobe surrounding the pale area of pars intermedia and degenerated neural lobe. Castration, hypophysectomy, and estrogen injection for periods up to 2 months had no effect on the size of the pituitary graft.

EVALUATION OF THE INFLUENCE OF PITUITARY GRAFTS ON HOSTS:

The functional activity of the ovaries and indirectly of the transplanted pituitary glands was evaluated by obtaining stained preparations of exfoliated vaginal cells from control mice and from mice bearing one or two pituitary grafts. In the normal mouse estrus occurs every 4½ days. Vaginal smears were obtained from 3 to 7 days preceding autopsy. Mice bearing one or two pituitary grafts usually displayed pseudopregnancy-like cycles. Mice without pituitary grafts showed cornified cells in the vaginal smears 45% of

the total number of days that they were smeared (see Table 2). The number of days during which a mouse showed cornified cells included the period of estrus as well as the days of late proestrus and early postestrus. Mice that had a pituitary transplant in place for 4 days showed cornified cells for 22% of the days during which smears were obtained. The percent of estrous type smears decreased to 15% at 14 days and 11% at 30 days after transplantation of the pituitary gland. Hypophysectomized, castrate, and hypophysectomized-castrate mice with pituitary grafts showed essentially no cornified vaginal cells. Estrogen-treated mice bearing pituitary transplants showed 56% days of cornified smears after 7 days of transplantation and 100% days of cornification after 30 days of transplantation. The pituitary transplant in hypophysectomized mice did not produce gonadotrophic hormones sufficient to maintain the normal reproductive cycle.

The ovaries were inspected and weighed at autopsy. The ovaries of mice bearing a pituitary transplant were slightly larger than those of intact controls and showed large corpora lutea. The ovaries of intact mice bearing one or two pituitary transplants were slightly heavier at 7 days and 2 months than the average weight of control ovaries, indicating some increased growth of corpora lutea, but this difference was not noted at 14 days and 1 month (see Chart 1). Ovaries of hypophysectomized mice quickly decreased in weight; after bearing a pituitary graft for one month, hypophysectomized mice had ovaries that weighed less than one-fourth that of intact ovaries from control

TABLE 2

PER CENT DAYS IN WHICH VAGINAL SMEARS SHOWED CORNIFIED CELLS FOR MICE WITH PITUITARY GRAFTS IN THE VARIOUS CATEGORIES

No. Days Grafts In Place	No. Mice	Days Smeared	Control Mice	Intact With Graft	Cast. With Graft	Hypoph. With Graft	Estrogen With Graft
0 days (control)	3	7	45%				
4 days	3	3		22%			
7 days	3	3		0%			56%
14 days	3	4		15%	0%	0%	
30 days	3	6		11%	0%	0%	100%
60 days	2	2		25%			

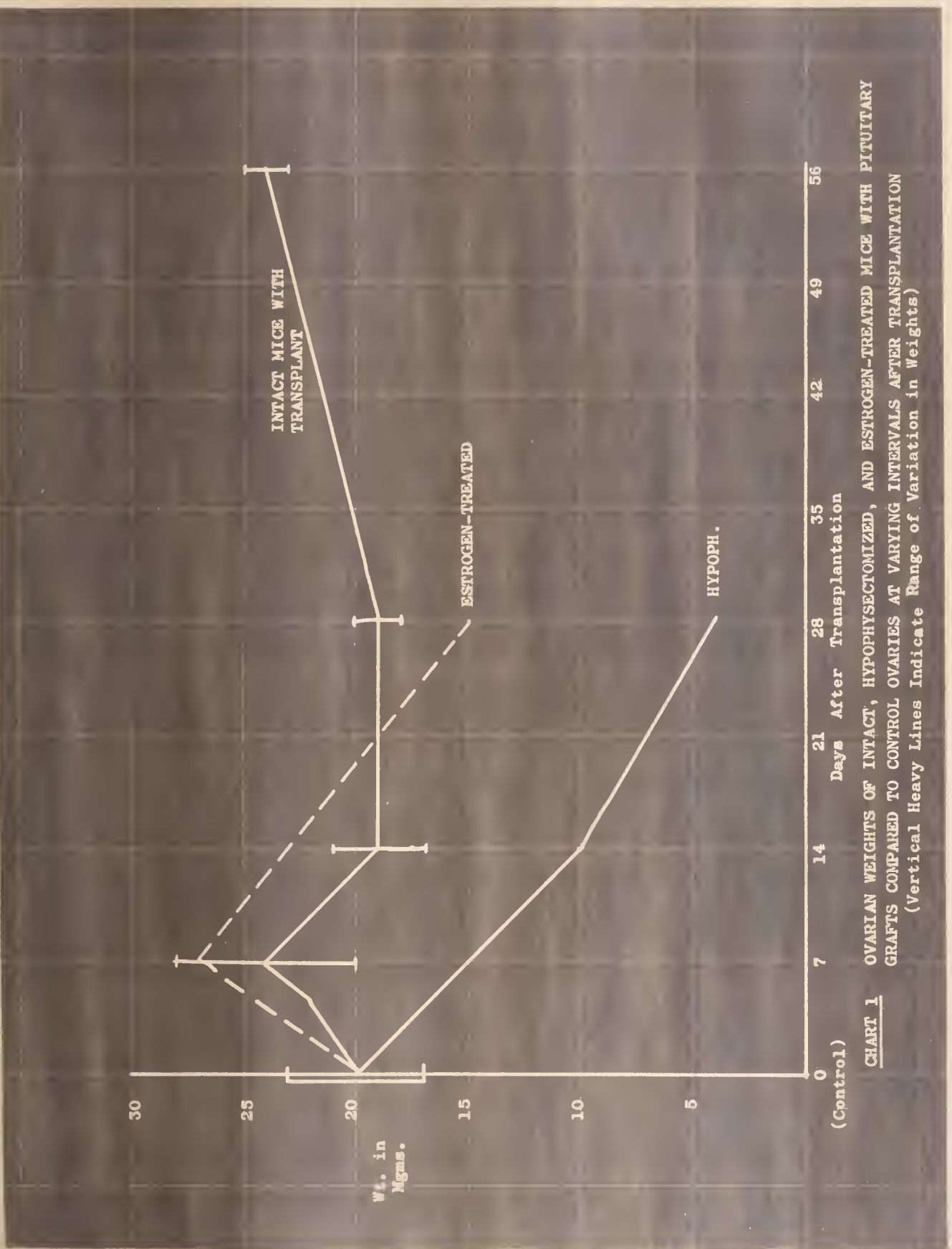


CHART 1 OVARIAN WEIGHTS OF INTACT, HYPOPHYSECTOMIZED, AND ESTROGEN-TREATED MICE WITH PITUITARY GRAFTS COMPARED TO CONTROL OVARIES AT VARYING INTERVALS AFTER TRANSPLANTATION
(Vertical Heavy Lines Indicate Range of Variation in Weights)

animals. The pituitary transplant in hypophysectomized mice did not maintain gonadotrophic function. Ovarian weights in mice bearing transplants and injected with estrogen for one month did not differ significantly from intact controls.

Microscopic examination of representative ovaries in normal control and in transplant-bearing mice was undertaken to evaluate the evidences of LTH secretion by the pituitary graft (see Figs. 1, 2). The ovaries of mice with pituitary grafts had larger corpora lutea and had more active corpora lutea than those of normal mice. Ovaries of graft-bearing mice had very few small antral follicles when compared with normal ovaries. Mice with grafts had fewer generations of corpora lutea and more active and large corpora luteal cells than mice without grafts. All of the ovarian reactions point to increased amounts of LTH stimulation in mice bearing pituitary transplants.

The uterus and cervix of each control and graft-bearing female mouse was weighed at autopsy (see Chart 2). The weights of uteri of mice with pituitary transplants did not differ significantly from the weights of control uteri. The uteri of castrate and hypophysectomized mice became atrophic very quickly and by 14 days they weighed one-third that of control uteri. Thus the pituitary graft in hypophysectomized mice failed to maintain uterine weight, even when ovaries were left in place. The uteri of estrogen-injected mice with transplants had weights several times that of animals that did not receive estrogen.

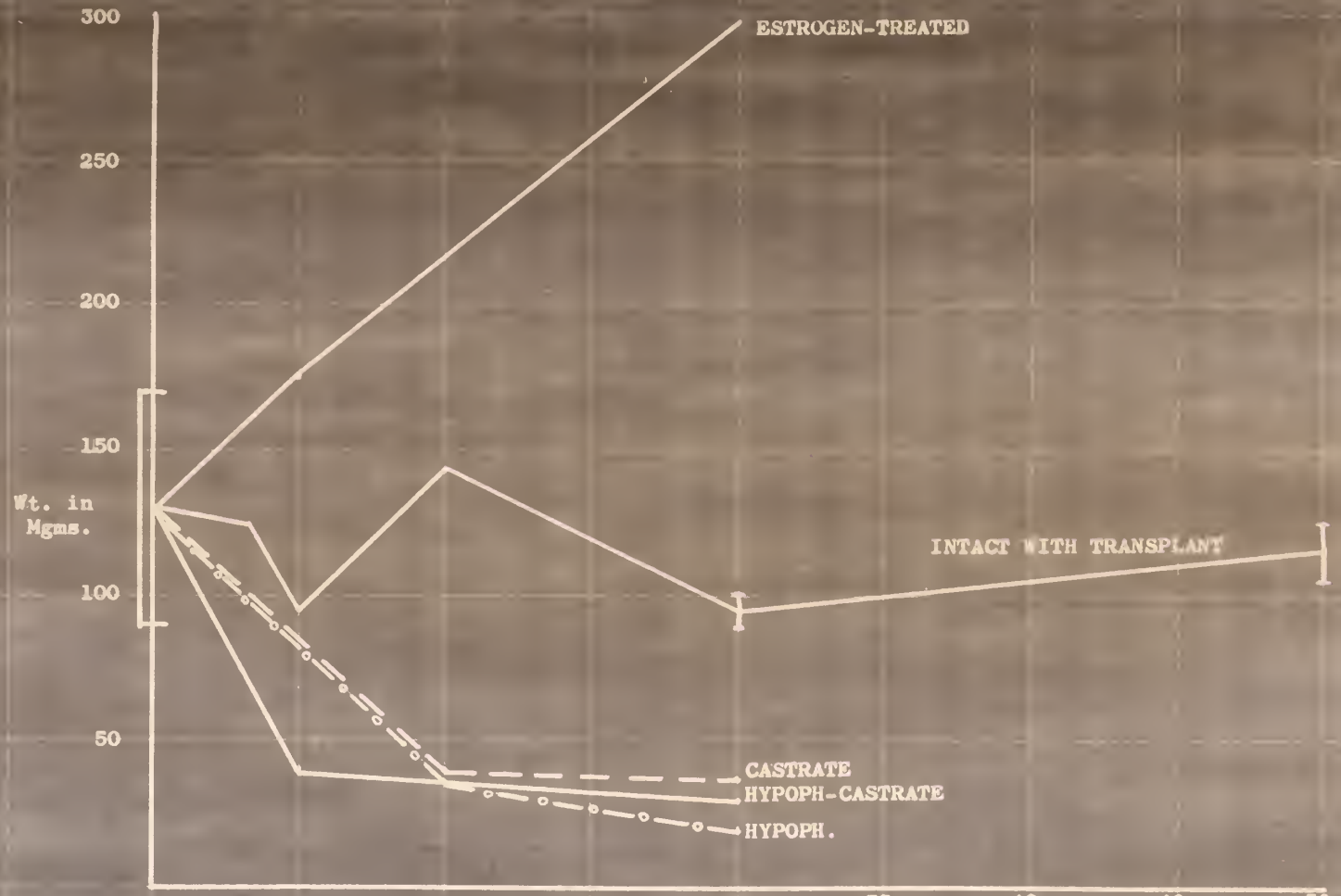


CHART 2 UTERINE WEIGHTS OF MICE WITH PITUITARY GRAFTS COMPARED TO CONTROL UTERI AT VARYING INTERVALS AFTER TRANSPLANTATION

(Vertical Heavy Lines Indicate Range of Variation in Weights)

The testes of untreated control mice, mice with pituitary transplants, and of hypophysectomized and estrogen-treated mice bearing pituitary transplants were weighed. The testes of mice bearing transplants weighed the same as those of the untreated mice (see Chart 3). Estrogen injection had no significant effect on the testicular weight of mice with transplants in place up to one month. The testes of hypophysectomized mice with transplants decreased markedly in weight; at one month the testes were less than one-fourth as heavy as those of control mice. Thus the pituitary graft did not maintain gonadotrophic function in hypophysectomized mice.

Seminal vesicles and prostate gland were weighed in all male mice at autopsy (see Chart 4). The range of weights was great because of the variable amount of secretion retained in the seminal vesicles. The intact mice with transplants and the estrogen-injected mice with transplants had seminal vesicle and prostate weights similar to those of control mice. The seminal vesicles of castrate and hypophysectomized mice were much smaller than those of normal animals, again indicating that the pituitary transplant was insufficient to maintain them.

The submaxillary glands were weighed from control mice, from male and female mice with transplants, and from castrate and hypophysectomized mice with transplants (see Charts 5,6). Weights of glands from control and transplant-bearing mice were similar, but hypophysectomized male and female mice with transplants had smaller submaxillary glands.

The possibility of somatotrophic function of the transplanted pituitary gland was evaluated in the hypophysectomized

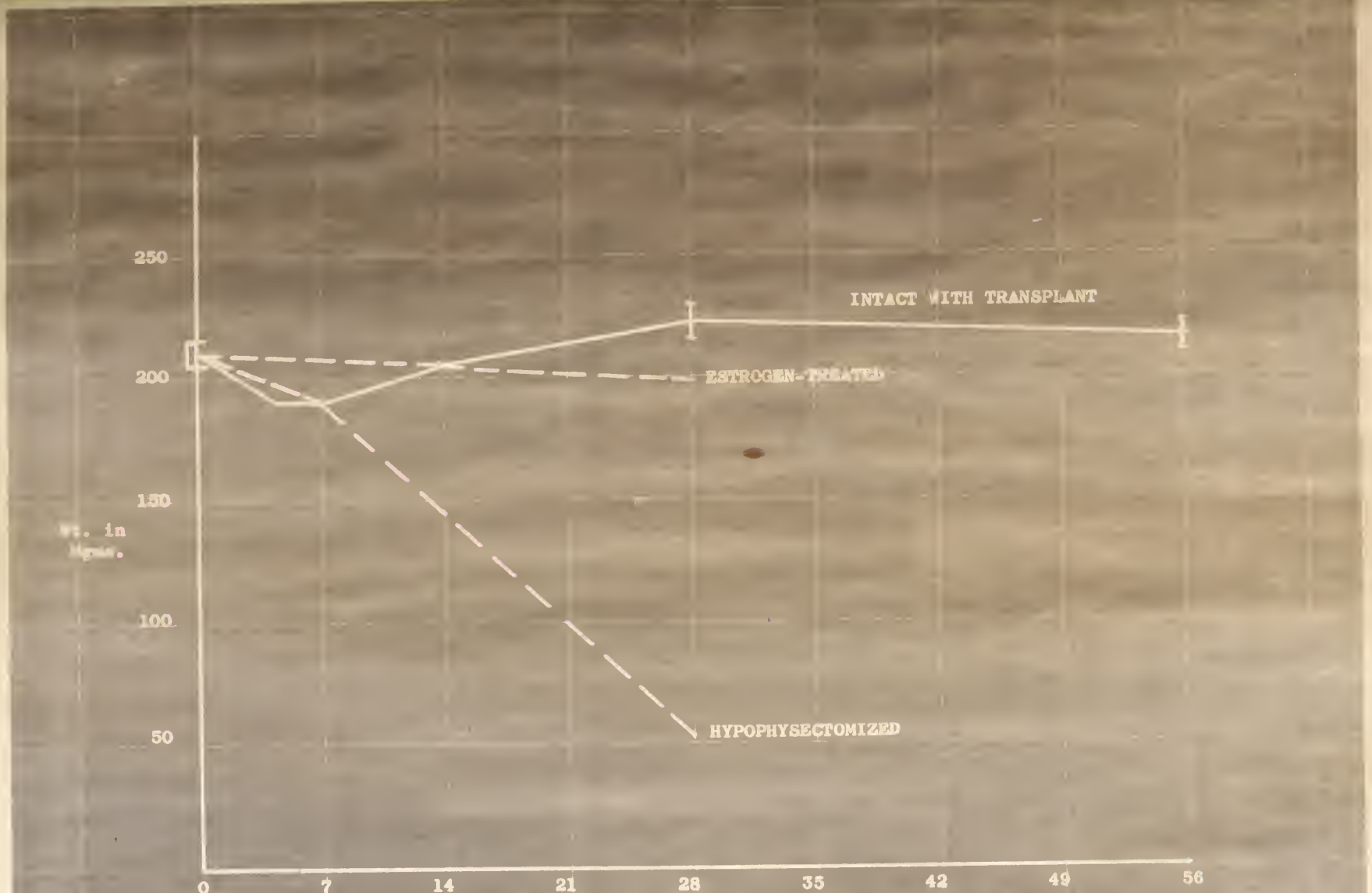


CHART 3 TESTICULAR WEIGHTS OF INTACT, HYPOPHYSECTOMIZED, AND ESTROGEN-TREATED MICE WITH PITUITARY GRAFTS COMPARED TO CONTROL TESTES AT VARYING INTERVALS AFTER TRANSPLANTATION

(Vertical Heavy Lines Indicate Range of Variation in Weight)

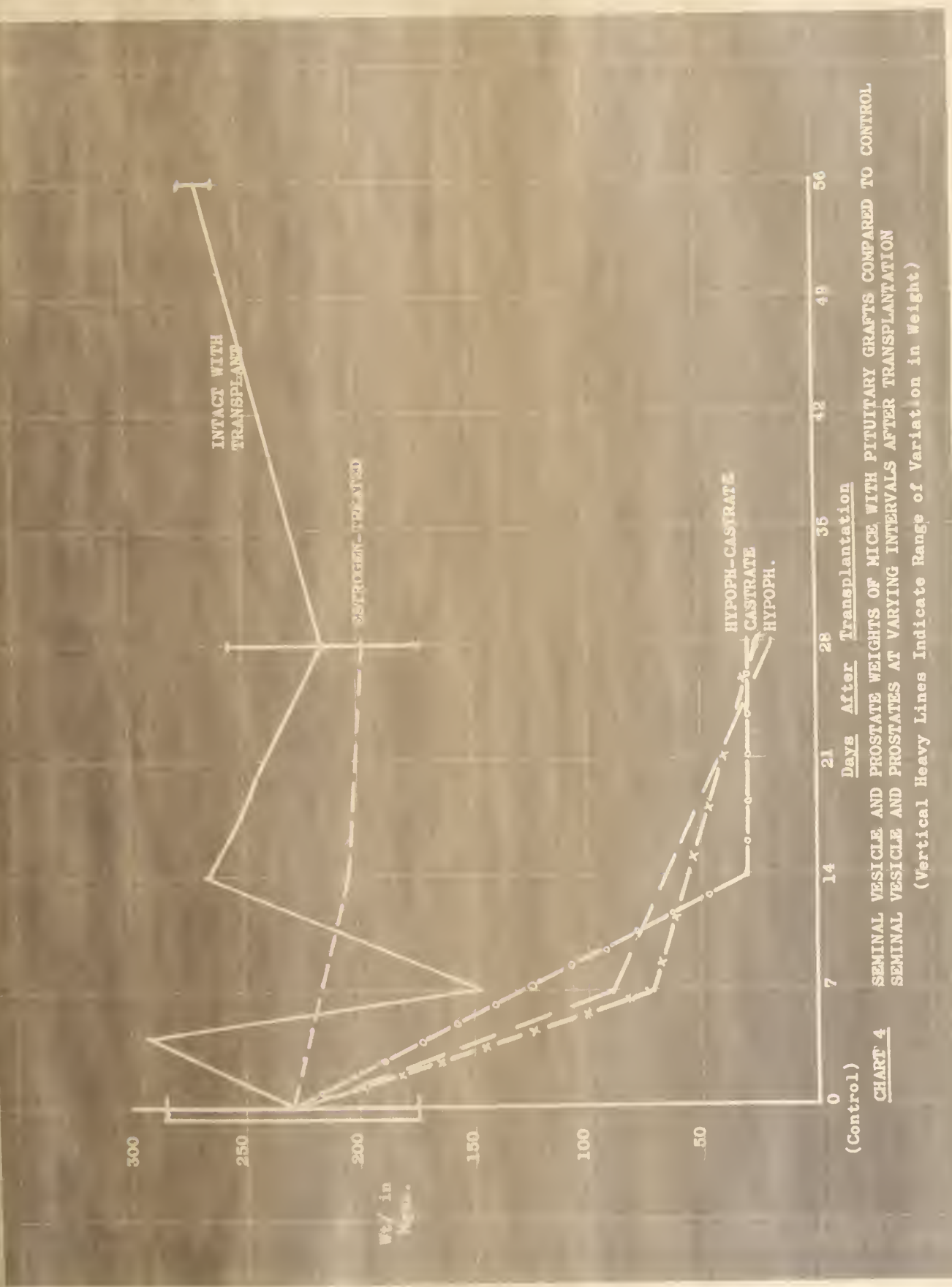


CHART 4 SEMINAL VESICLE AND PROSTATE WEIGHTS OF MICE WITH PITUITARY GRAFTS COMPARED TO CONTROL SEMINAL VESICLE AND PROSTATES AT VARYING INTERVALS AFTER TRANSPLANTATION (Vertical Heavy Lines Indicate Range of Variation in Weight)

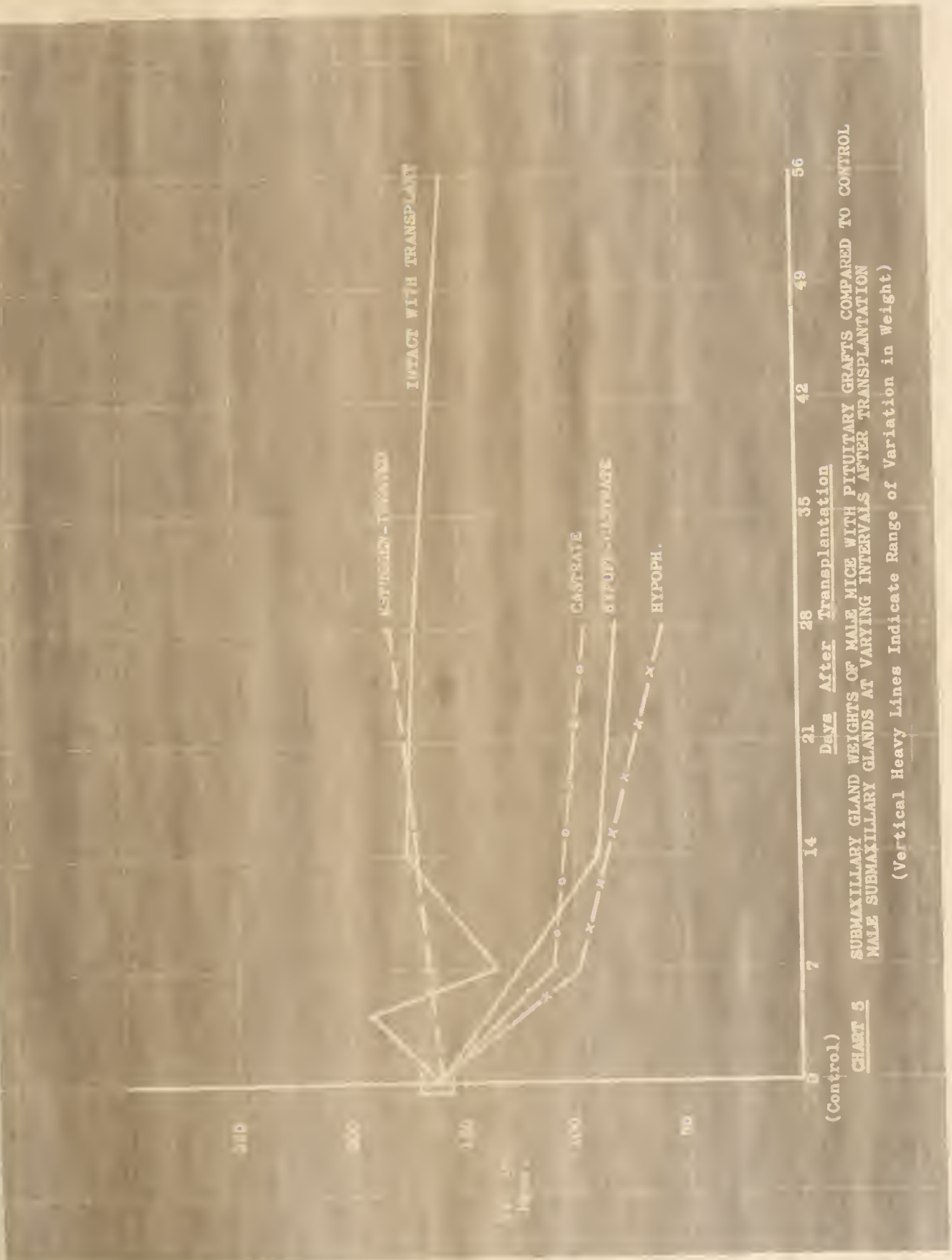


CHART 5 SUBMAXILLARY GLAND WEIGHTS OF MALE MICE WITH PITUITARY GRAFTS COMPARED TO CONTROL MALE SUBMAXILLARY GLANDS AT VARYING INTERVALS AFTER TRANSPLANTATION

(Vertical Heavy Lines Indicate Range of Variation in Weight)

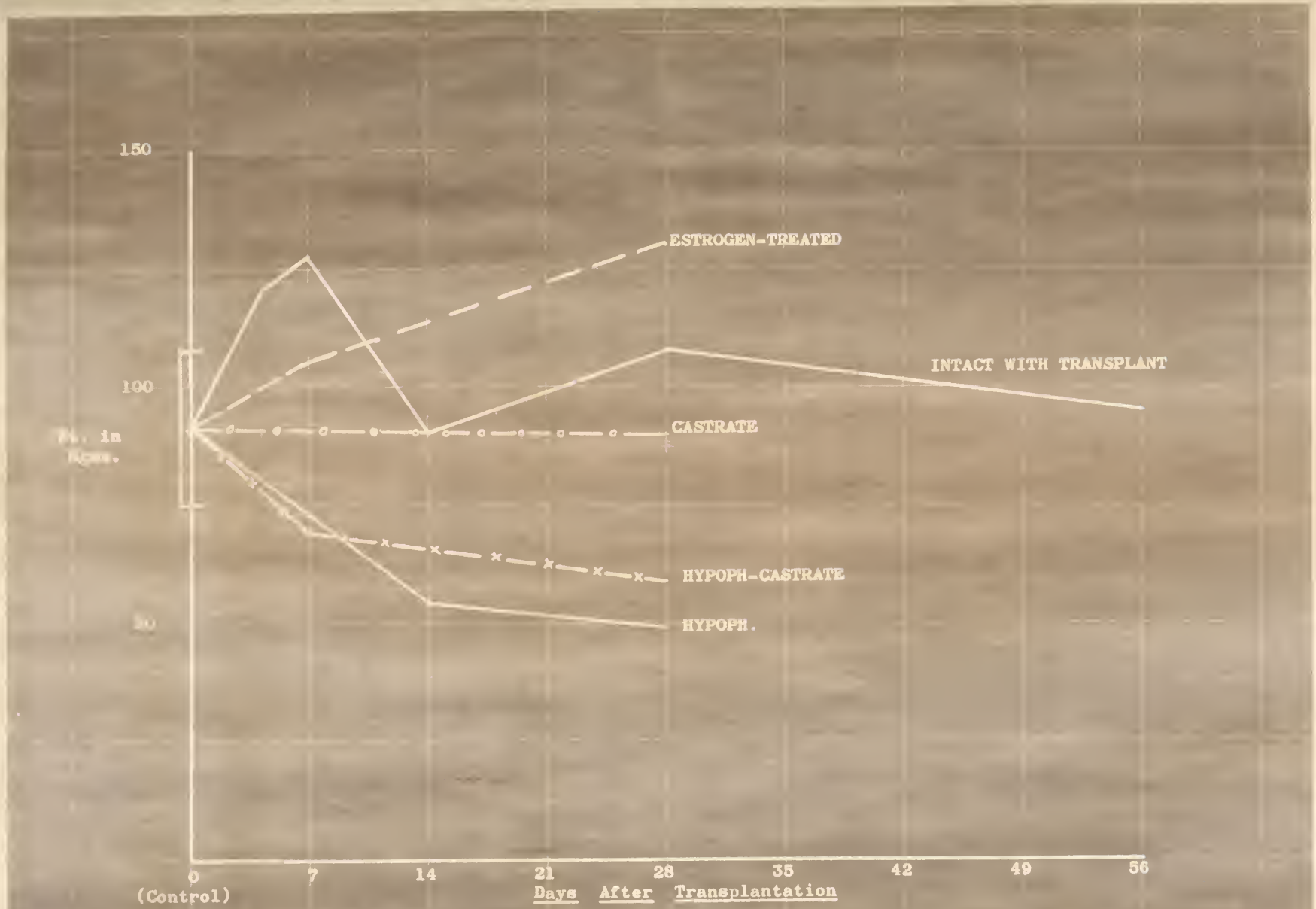


CHART 6 SUBMAXILLARY GLAND WEIGHTS OF FEMALE MICE WITH PITUITARY GRAFTS COMPARED TO CONTROL FEMALE SUBMAXILLARY GLANDS AT VARYING INTERVALS AFTER TRANSPLANTATION

(Vertical Heavy Lines Indicate Range of Variation in Weight)

male and female mice with pituitary transplants. Male and female mice were weighed and hypophysectomized on one day, received a pituitary transplant on the following day, and were weighed daily until the time of autopsy. Hypophysectomy resulted in the loss of one to two grams of body weight one day after the operation. Transplantation of one or two pituitary glands subcutaneously one day after hypophysectomy resulted in an average increase of one to three grams of body weight in the few days after transplantation, possibly as a result of the release of hormones from the grafted gland. At autopsy, however, all male and female hypophysectomized mice with transplants had lost weight (see Table 3). Weights of male mice that had been hypophysectomized or hypophysectomized and castrated decreased 15 percent by 30 days after hypophysectomy and after the pituitary had been in place for 30 days. Females showed a 5 to 9% loss of body weight under similar conditions.

The kidneys of hypophysectomized male and female mice were weighed at autopsy. The kidneys decreased in size markedly after hypophysectomy (see Chart 7), in spite of the presence of the pituitary grafts. This indicates that the pituitary graft did not maintain the kidneys as the intact pituitary gland does.

EVALUATION OF THE EFFECTS OF PITUITARY TRANSPLANTS ON THE MAMMARY GLANDS:

Subcutaneous pituitary grafts in intact female mice quickly and profoundly stimulated the adjacent and peripheral

TABLE 3

PERCENT WEIGHT LOSS OF HYPOPHYSECTOMIZED MICE WITH GRAFTS AT AUTOPSY COMPARED TO PRE-HYPOPHYSECTOMIZED WEIGHT

CATEGORIES OF HYPOPH. MICE WITH PIT. GRAFTS	% Weight Loss After Graft In Place For		
	7 days	14 days	30 days
Male Hypoph. with graft	6.9%		14.8%
Male Hypoph-Cast. w. gr.		3.6%	15.4%
Female Hypoph. w. graft		9.1%	9.1%
Female Hypoph-Cast. w. gr.	6.5%		4.5%

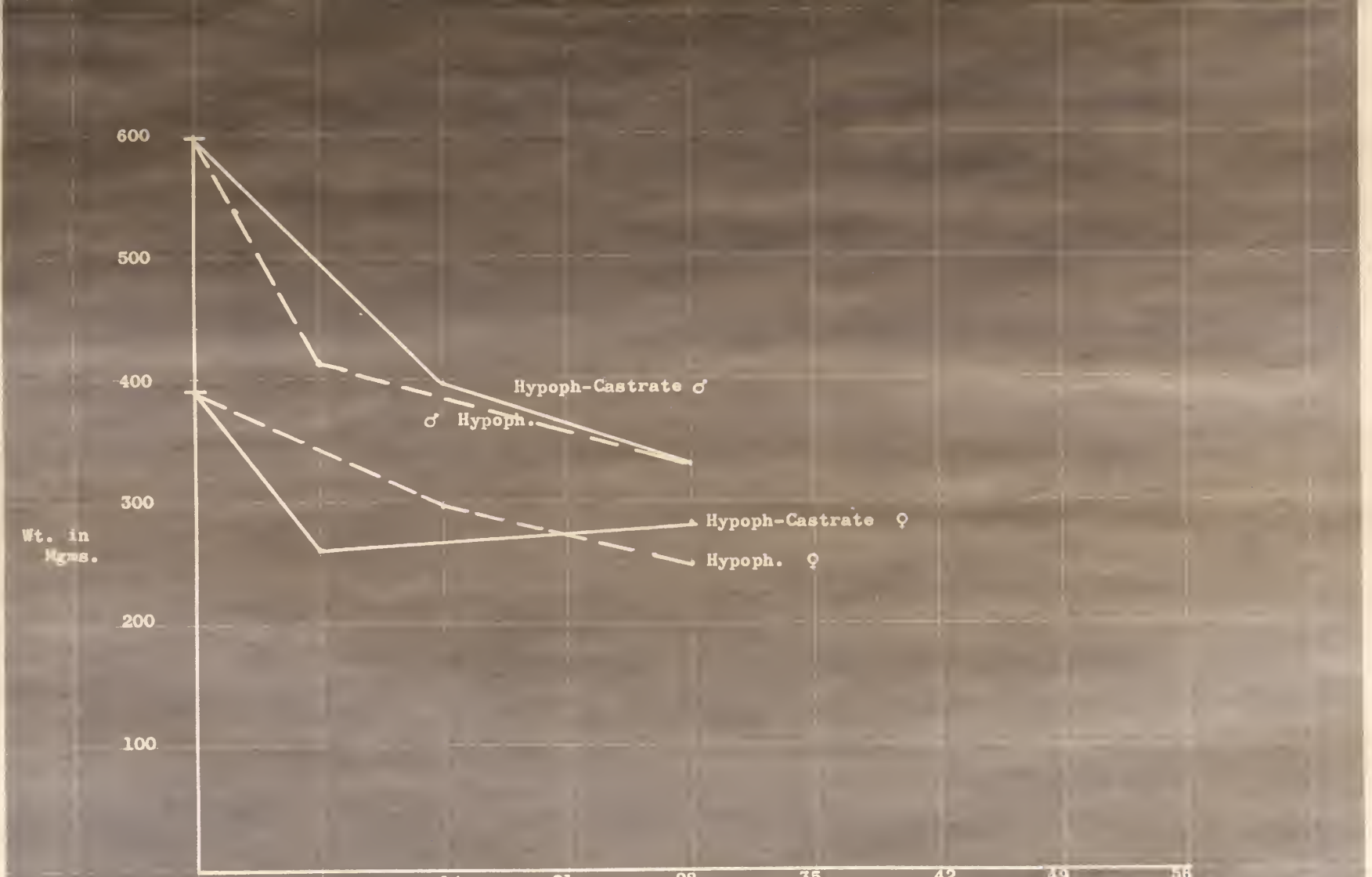


CHART 7 KIDNEY WEIGHTS OF MALE AND FEMALE HYPOPHYSECTOMIZED AND HYPOPHYSECTOMIZED-CASTRATED MICE WITH PITUITARY GRAFTS COMPARED TO CONTROL KIDNEY WEIGHTS AFTER TRANSPLANTATION

mammary glands. In male mice, pituitary grafts had no gross or microscopic effect on the development of the mammary glands, even after the graft had been in place for 2 months. In female mice, the pituitary graft produced local alveolar and ductal stimulation in the immediate area of the graft, as well as stimulation of the mammary glands at a distance or on the opposite side from the pituitary transplant (see Table 4). Mammary glands from adult, non-pregnant, non-lactating female mice without transplants showed minimal alveolar and ductal development, rating 1 on a scale from 0 to 4 (see page 29) (see Fig. 3).

Four Days After Transplant: Mammary glands of intact female mice with one pituitary graft placed subcutaneously on the right side showed a marked alveolar stimulation (see Fig. 4). Mammary glands 2 and 3 on the right side were rated 2, while those on the left side where there was no graft rated 1/. No milk or milk-like substance was noted in the ducts.

Seven Days After Transplant: Intact female mice with one graft on the right side and one on the left showed even more marked stimulation. Glands in the immediate area of the graft showed an intense local stimulation and in addition the more peripheral mammary glands were also stimulated (see Fig.5). The localization of the mammotrophic effectis exemplified by the detailed observation on this mouse. The pituitary graft was recovered from the substance of the right third mammary gland. Immediately around the graft the gland shows

TABLE 4

DEVELOPMENT OF MAMMARY GLANDS OF FEMALE MICE WITH PITUITARY GRAFTS

No. Days Grafts in Place	Mouse #	No. of Pituitary Grafts	Development of Glands*		Ducts Contain Milk
			Right	Left	
0 days (control)	#1	0	1	1	No
4 days	#6	1 right 0 left	2	1/	No
7 days	#21	1 R 1 L	2 (areas of 1/ & 3)	2	No
14 days	#76	1 R 1 L	3 (areas of 2/)	3	No
30 days	#46	1 R 1 L	3 (areas of 2/)	2/	Yes
60 days	#57	1 R 0 L	2/ (areas of 3)	2	Yes
Pregnant	#50	1 R 0 L	2/ (areas of 3)	--	Yes
3 Day Post-Partum	#49	1 R 0 L	4/	--	Yes

* 0= extreme atrophy; 1 = only main and interlobar ducts;...; 4 = full lactation.

a stage of development rated at 3, particularly around the main duct of the gland. At the periphery the stage of gland development was rated at 1/; thus, an overall rating of 2 was assigned to the glands of the right side. The second mammary gland was at some distance from the graft and showed a uniform rating of 2.

Fourteen Days After Transplant: Intact female mice with one pituitary graft on each side showed marked mammary stimulation on both sides, rating 3 (see Fig. 6). There was still no milk or milk-like substance observed in the ducts grossly. There was still minor non-uniformity noted in the development of the glands in direct approximation to the graft, but mammary stimulation was so extensive that these local differences were less marked.

One Month After Transplant: Intact female mice with one pituitary graft on each side showed intense mammary stimulation with milk or milk-like substance in all ducts of the mammary glands (see Fig. 7). Female mice that had been castrated and received a pituitary transplant one month previously showed atrophy of the mammary glands, rated at 1-, and thus essentially no local or peripheral effects of the pituitary graft (see Fig. 8). Female mice that had been hypophysectomized and received a pituitary transplant one month previously showed extreme atrophy of the mammary glands, rated at 0, and thus the graft had no mammotrophic effect on the animal (see Fig. 9). Female mice that had a transplant for one month and received estrogen for that period showed considerable

mammary development, rated at 2/ (see Fig. 10).

Two Months After Transplant: Intact female mice with one pituitary graft on the right side and no pituitary graft on the left showed extensive mammary development, rated at 2 to 3, and all ducts were filled with milk or milk-like substance (see Fig. 11). Glands of the right side showed more development than those of the left because the pituitary graft was present on the right only. All glands, however, had milk-filled ducts.

Pregnant mice bearing a pituitary transplant on the right side only during pregnancy showed 2/ to 3 mammary development in all glands with milk-filled ducts. Mice that were autopsied 3 days post-partum and that bore a pituitary transplant on the right during pregnancy showed profuse mammary development and milk-production, rated at 4/.

MICROSCOPIC EVALUATION OF PITUITARY GLAND TRANSPLANTS:

General Characteristics of Transplanted Pituitary Glands:

Pituitary glands transplanted subcutaneously in mice showed a massive central infarct the first day after transplantation. Histologically normal pituitary cells were confined to the outer margin of the graft (see Figs. 12, 13). Twenty-four hours after transplantation a layer of 3-4 normal appearing pituitary cells surrounded a large area of necrotic and degenerating cells. The degenerating area contained many polymorphonuclear leucocytes and mononuclear cells, and cell borders were indistinct and nuclei were pyknotic. No mitoses were seen at 24 hours. Many acidophilic and

basophilic cells were identified in the outer layer of cells. No vascular proliferation could be identified at this early time, but sinusoids appeared engorged with erythrocytes.

After four days of transplantation the pituitary grafts still showed the extensive central necrosis (see Fig. 14). In the necrotic area of the graft, cell boundaries were indistinct, nuclei were pyknotic, and many inflammatory cells were present. Several mast cells were noted in the grafts. A layer of normal-appearing cells remained at the periphery of the graft and several mitotic figures were identified among the pituitary cells. Fibroblastic proliferation was evident at the borders of the graft and fibroblasts extended into the cords of pituitary cells. A vascular response was evident; capillary growth extended from the edges to the more central areas of the graft. A few areas of pars intermedia cells were seen, but no remnant of the neural lobe could be identified.

After seven days of transplantation the grafts showed beginning fibrosis and resolution of the necrotic center (see Fig. 17). The inflammatory response was diminished and fibroblastic proliferation was invading the necrotic portions of the graft. Several mast cells were again identified. The intraglandular cleft between anterior lobe and pars intermedia was still evident in many grafts. A few mitotic figures were seen in pituitary cells. Capillaries had infiltrated most areas of the graft and they contained many erythrocytes. The pars intermedia appeared increased

in size in many of the grafts of this age. Pars intermedia cells were arranged in clusters in many of the grafts and the cells were large and distinct (see Figs. 20, 21). No neural lobe tissue could be identified.

After fourteen days of transplantation the pituitary grafts showed almost complete resolution of the necrotic areas with no inflammatory response and considerable central scarring with apparent contracture and decrease of the size of the graft. (see Fig. 22). No mitoses were seen in the pituitary cells. The intraglandular cleft was apparent in many sections. The pars intermedia was large and the cells were arranged in clusters or whorls. Vascularization was extensive and extended throughout the graft.

By the end of one month the transplants showed central scarring and essentially complete organization of the graft (see Figs. 26, 28). The pars intermedia was large and cells were arranged as previously described. At the end of two months the graft appeared identical to that at one month.

CYTOLOGICAL AND HISTOCHEMICAL CHARACTERISTICS OF TRANSPLANTED PITUITARY GLANDS:

After the pituitary transplant had been in place subcutaneously in intact mice for 4 days, basophilic and acidophilic cells were frequent and readily recognized (see Figs. 14, 15, 16). Periodic acid Schiff (PAS) positive cells were numerous and stained intensely; they were not as numerous as in the normal pituitary gland. The PAS staining was present predominently in clumped granules within the cytoplasm of the

basophiles. No distinction between gonadotrophs and thyrotrophs could be made. The identifiable basophiles and acidophiles were present in only that part of the graft that was not undergoing necrosis. Numerous acidophiles were present in the graft and stained intensely with Luxol Fast Blue (LFB). Nuclei of the acidophiles were distinct and were identical to those of the normal pituitary gland. The basophiles and acidophiles were as large as those in the normal pituitary gland (see Chart 8). Chromophobes were abundant and were similar to those of the normal pituitary. Pars intermedia cells were relatively few in number and took very little PAS stain.

Grafts that had been extracted in 2.5% TCA contained well-stained LFB positive cells and weakly stained PAS positive cells (see Page 25). This technique did not reveal any valuable findings so it will not be commented on in further descriptions.

Grafts that had been extracted in 0.5% TCA contained many LFB-positive cells and no PAS-positive cells (see Page 24). This finding was in general true for all grafts that were extracted in 0.5% TCA; when the sublimate-formol fixed graft showed acidophiles, the TCA-extracted graft also showed acidophiles in equal numbers and staining intensity. Also, as will be shown later, 0.5% TCA does not appear to differentiate reliably between acidophiles containing LTH and those containing other acidophilic hormones. This concentration of TCA does, however, extract all PAS-positive material from the pituitary

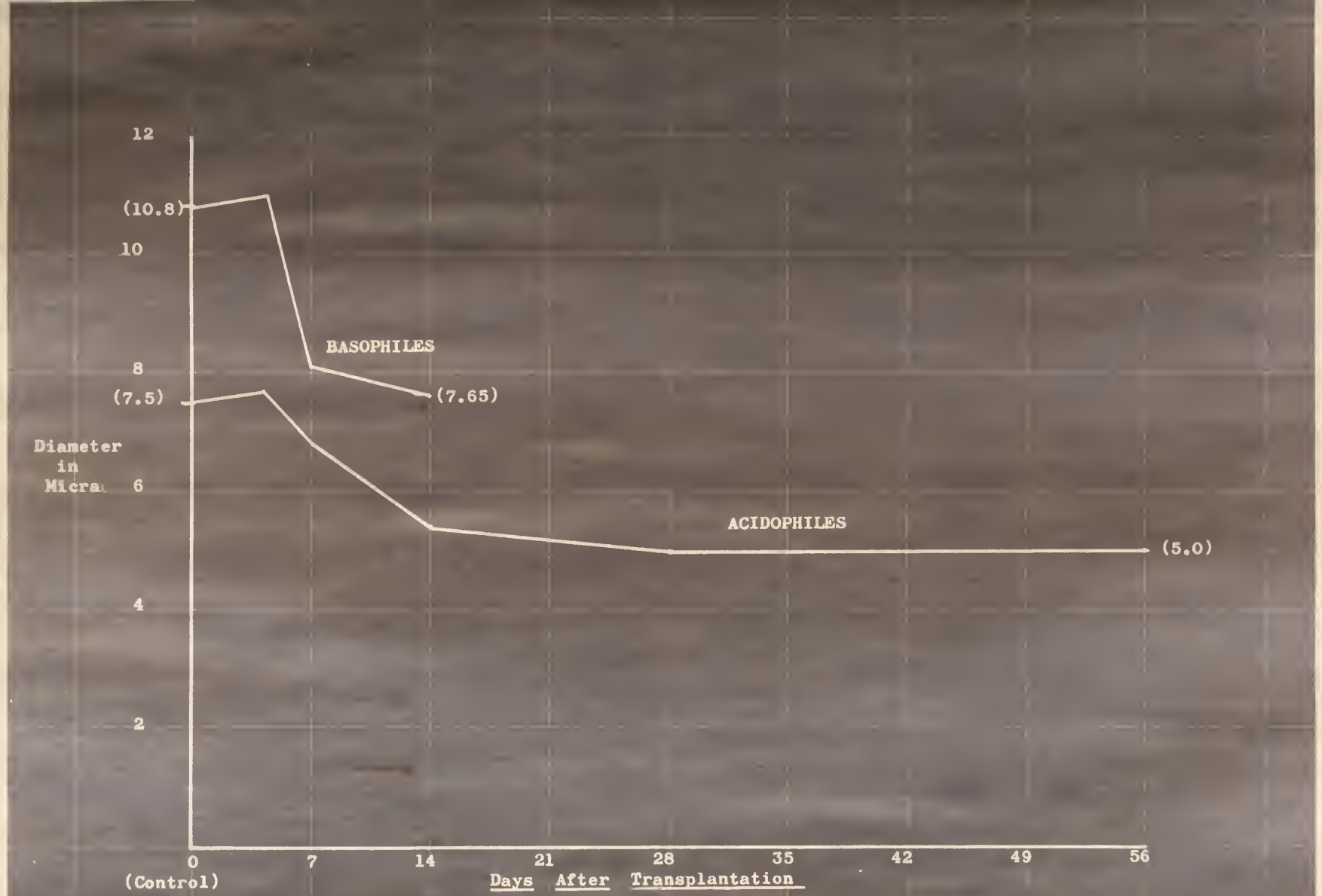


CHART 8 AVERAGE DIAMETERS OF BASOPHILES AND ACIDOPHILES IN PITUITARY GLANDS TRANSPLANTED SUBCUTANEOUSLY INTO INTACT MICE AT VARYING INTERVALS AFTER TRANSPLANTATION

glands and pituitary transplants. Therefore, this technique will not be described for each category and time of transplantation.

In intact male and female mice with a pituitary graft in place for 7 days the transplant again showed numerous acidophiles and basophiles (see Figs. 17, 18, 19). Basophiles showed a moderately intense reaction with PAS, but they had decreased in size (see Chart 12). The average basophiles in the normal pituitary measured 10.8 micra in the widest diameter, but in the 7-day grafts they measured approximately 8 micra in diameter. Acidophiles were also numerous and stained intensely with LFB. The acidophiles were also smaller than those of normal pituitary glands (see Chart 12), measuring 7.5 micra in the normal pituitary and 6.8 micra in the grafts. Chromophobes were abundant and were identical to those of the normal pituitary gland. No distinction between gonadotrophs and thyrotrophs could be made in the PAS-positive cells on the basis of cellular morphology. The transplanted gland generally lost its differentiation into sex zone, central zone, etc., that is present in the normal pituitary.

The pars intermedia was larger in the 7-day grafts (see Figs. 20, 21). The cells of the pars intermedia were large and were arranged in whorls and clusters in different portions of the graft. Many of the cells stained lightly with PAS as do cells of the pars intermedia in the intact gland. The negative image of the Golgi apparatus was evident in some cells of the pars intermedia.

Pituitary grafts from male hosts that had been castrated and transplanted 7 days previously showed numerous and intensely stained PAS-positive and LFB-positive cells. No castration cells could be identified in these grafts.

Pituitary grafts from male hosts that were hypophysectomized and grafted for 7 days contained intensely stained PAS cells. Although most of the acidophiles stained lightly with LFB, a few darkly staining acidophiles were seen.

Pituitary grafts from estrogen-treated female mice bearing transplants for 7 days had lightly stained PAS basophiles that were fewer in number than basophiles in untreated hosts. Numerous acidophiles present in the graft stained lightly with LFB.

Pituitary transplants that had been in place for 14 days in intact male and female mice showed moderate numbers of basophiles and numerous acidophiles (see Figs. 22, 23). The PAS-positive cells were small, averaging 7.6 micra in the widest diameter whereas basophiles in intact glands averaged 10.8 micra. The PAS material was present in sparse, coarse granules staining rather lightly, but many cells of this type were seen. Acidophiles were numerous, although they measured 5.3 micra in diameter in comparison to 7.5 micra for acidophiles in intact glands. LFB stained the acidophiles with moderate intensity. Chromophobes were unremarkable.

The pars intermedia was large in these grafts and many of the cells stained intensely with PAS while others remained relatively unstained (see Figs. 24, 25). In general, the darkly stained PAS cells were smaller than the larger, more

lightly stained intermedia cells. Golgi images were more frequent, particularly in the large, lightly stained cells.

The cellular characteristics of transplants in castrated, hypophysectomized, and estrogen-treated mice did not differ significantly from the grafts in the intact mice bearing grafts for 7 days.

Pituitary transplants that had been in place for 1 month in intact male and female mice showed few basophiles and more numerous acidophiles (see Figs. 26, 27). PAS-positive cells, when present, stained lightly and had few cytoplasmic granules. The numbers of acidophiles ranged from moderate to numerous. They were about 5.0 micra in diameter, approximately as small as those at 14 days. Some acidophiles stained as intensely and uniformly with LFB as those of the normal pituitary, but others had foamy-appearing cytoplasmic LFB-positive material. Chromophobes were numerous and were unremarkable.

The pars intermedia was similar to that at 14 days following transplantation (see Fig. 28). Cells of the pars intermedia stained with PAS, some intensely and some much more lightly. The darker cells were smaller than the light-staining cells, and also more frequently contained Golgi images.

Pituitary grafts were examined from male and female mice that had been castrated and transplanted 1 month previously. A few sparsely granulated cells stained lightly with PAS, and numerous acidophiles stained with moderate to intense LFB (see Fig. 29). These grafts did not differ from those in intact hosts 1 month after transplantation.

Pituitary grafts from hypophysectomized male and female mice were examined after 1 month (see Fig. 30). PAS-positive cells were rare, and when present had few lightly stained granules. The small acidophiles were moderately numerous and moderately to intensely stained with LFB. These grafts did not differ from those in intact hosts 1 month after transplantation.

Pituitary grafts in estrogen-treated male and female mice for 1 month contained very few lightly-stained basophiles (see Fig. 31). Acidophiles were numerous and somewhat larger than the acidophiles in grafts of intact animals. The LFB stained the smaller acidophiles intensely, but other acidophiles had foamy-appearing cytoplasmic LFB-positive material.

Pituitary glands transplanted for 2 months in intact male and female mice were similar to the grafts described after transplantation for 1 month. (Fig. 32, 33). PAS-positive cells were rare, and when present, had few very lightly stained granules. LFB-positive acidophiles were moderate in number and were small, measuring about 5 micra. They stained with moderate intensity and some of the larger cells had foamy-appearing cytoplasmic LFB-positive material.

The pars intermedia in the grafts was similar to that at 1 month following transplantation and was composed of cells staining darkly with PAS and larger, more lightly staining cells (see Fig. 34).

HISTOLOGIC EVALUATION OF PITUITARY GLANDS OF MICE BEARING PITUITARY TRANSPLANTS:

The pituitary glands of host animals were examined with the PAS-LFB stains and were compared with the glands of mice without transplanted pituitary glands. Cell counts of the pituitary glands were done to enable comparison of the relative numbers of cells in the glands of mice of the different groups (see Charts 9, 10).

Pituitary glands of normal adult female mice showed 39% acidophiles, 13% basophiles, and 48% chromophobes. Pituitary glands of normal adult male mice had 59.5% acidophiles, 22.5% basophiles, and 18% chromophobes. The basophiles of the intact female pituitary gland were stained less intensely with PAS than were those of the male. Acidophiles stained equally in intensity. The distribution of the cell types (see Figs. 35, 36, 37) corresponded to the usual pattern (see Pages 3, 6).

The pituitary glands in female mice bearing a pituitary transplant for from four to 56 days showed approximately equal numbers of basophiles and acidophiles as those of the control pituitary glands (see Chart 9). The numbers of basophiles and acidophiles in the host animals were seemingly not affected by the presence of the pituitary graft. The relative intensities of staining with PAS and LFB were the same in host and control pituitaries.

Male mice bearing a pituitary transplant for from 4 to 56 days showed equal numbers of basophiles in host and control animals, both having approximately 23-24% basophiles (see Chart 10). Pituitary glands of host mice had fewer acidophiles 2 months after transplantation, however, than did

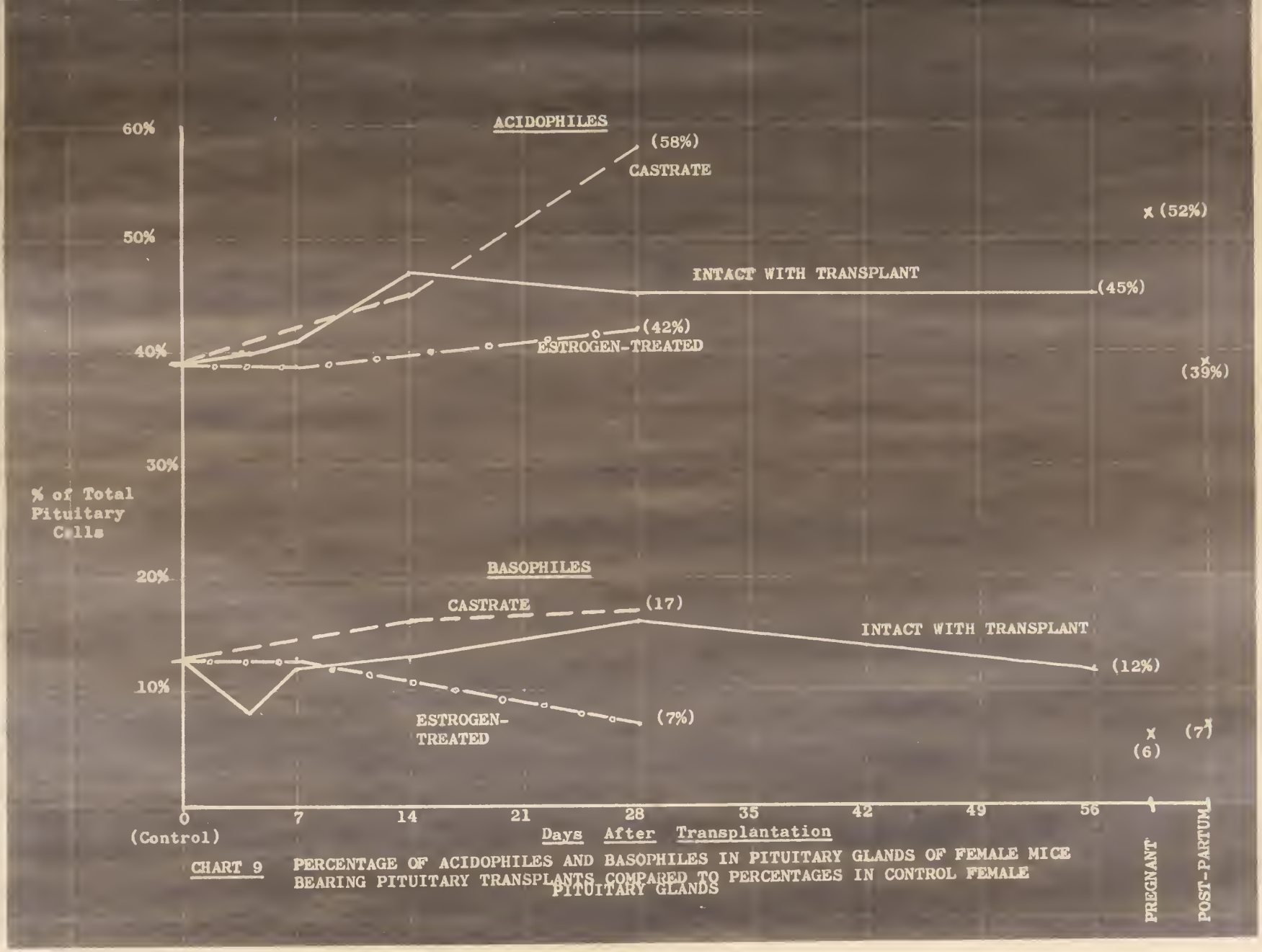


CHART 9 PERCENTAGE OF ACIDOPHILES AND BASOPHILES IN PITUITARY GLANDS OF FEMALE MICE BEARING PITUITARY TRANSPLANTS COMPARED TO PERCENTAGES IN CONTROL FEMALE PITUITARY GLANDS

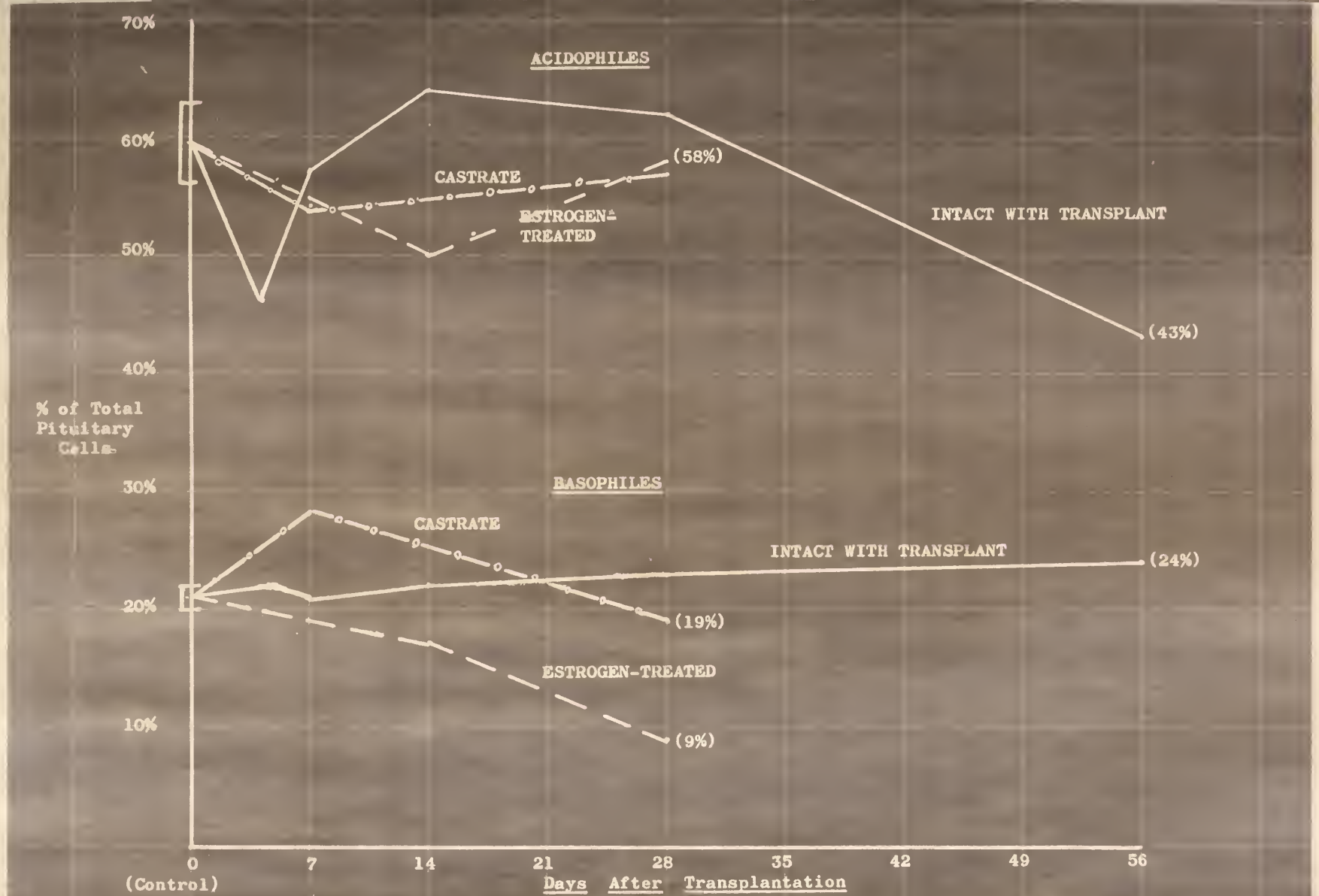


CHART 10 PERCENTAGE OF ACIDOPHILES AND BASOPHILES IN THE PITUITARY GLANDS OF MALE MICE BEARING PITUITARY TRANSPLANTS COMPARED TO PERCENTAGES IN CONTROL MALE PITUITARY GLANDS

those of control animals. Glands of host mice had 62% acidophiles at 1 month and 43% at 2 months as compared to 60% in pituitary glands of control male mice, but this may have been artifactual since the 2 month count was based on only one pituitary gland. Both PAS and LFB chromophilic cells stained with equal intensity in transplant-bearing and control mice.

The pituitary glands of castrated hosts bearing a transplant for 1 month contained castration cells. The castration cells stained lightly with PAS and a clear vacuole filled many of these cells. Castration did not significantly change the number of basophiles in male and female mice bearing grafts for one month, the number of basophiles being equal to the number in control animals if castration cells were included (see Charts 9, 10).

Estrogen-treated male and female mice bearing pituitary transplants for 1 month had many fewer basophiles than control pituitary glands (see Charts 9, 10). PAS stained the basophiles very lightly; otherwise, no other cellular characteristics were noted that differed from those of basophiles in untreated mice. The number of acidophiles was not changed in estrogen-treated host mice.

The percentage and intensity of staining of acidophiles of intact pituitary glands that were fixed in sublimate-formol were compared with similar qualities of the acidophiles of glands that were extracted in 0.5% TCA in an attempt to determine if differences in the counts existed that could support the hypothesis that 0.5% TCA allows only those

acidophiles that contain LTH to be stained (see Page 8). The percentage of acidophiles in the pituitary glands of female mice that stained with LFB after extraction with 0.5% TCA was relatively the same as the non-extracted glands (see Chart 11). The percentage of acidophiles in pregnant and 3-day post-partum mice was approximately equal in 0.5% TCA extracted and non-extracted pituitary glands, where LTH content should be greatest. In male mice bearing pituitary transplants for 2 months the number of acidophiles in 0.5% TCA extracted and non-extracted pituitaries were approximately equal (see Chart 12). These observations tend to discount the hypothesis (see Page 8) that LTH is contained in only a few acidophiles, if one assumes, of course, that LTH of the mouse is similar to that of the rat.

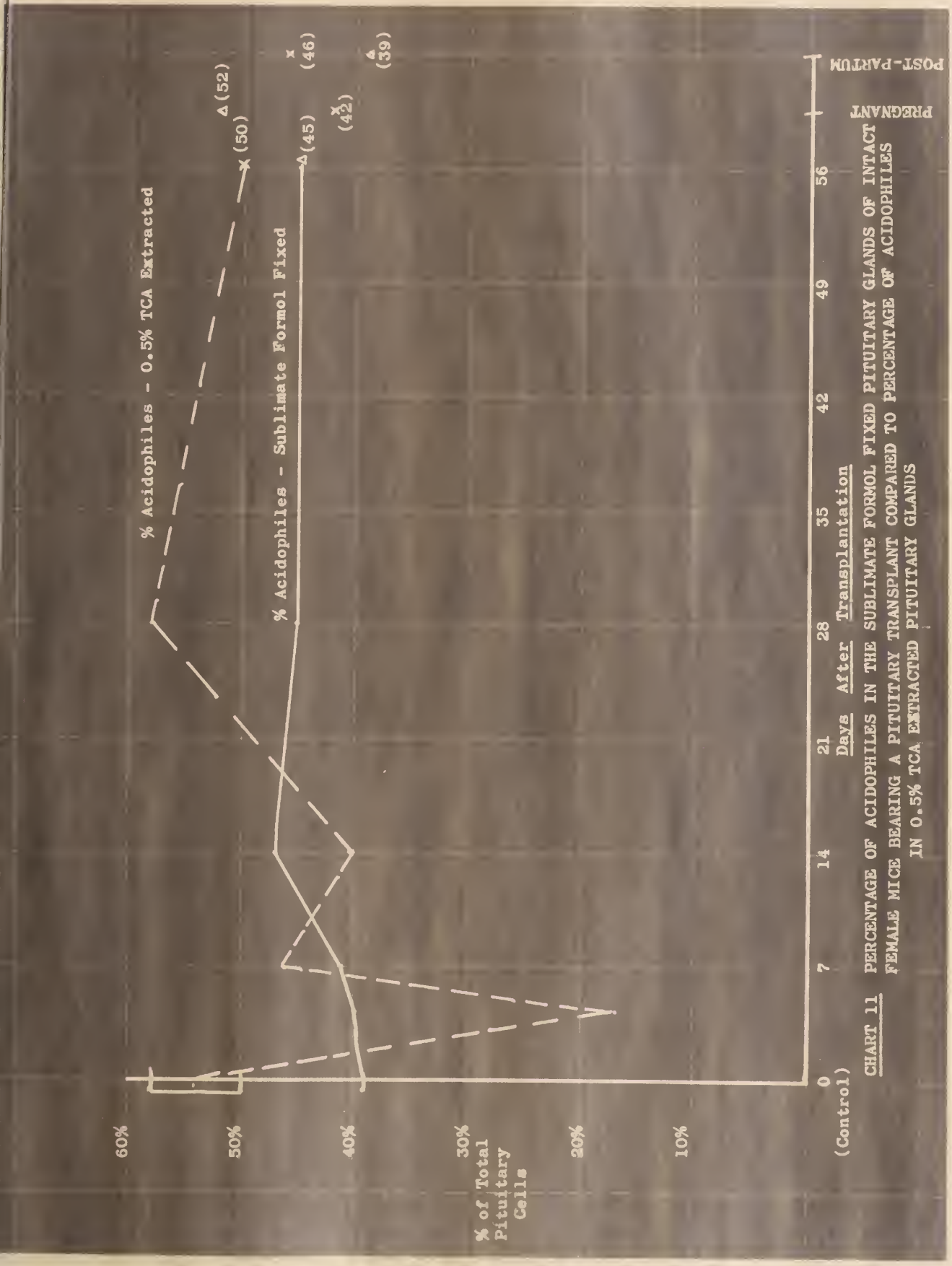


CHART 11 PERCENTAGE OF ACIDOPHILES IN THE SUBLIMATE FORMOL FIXED PITUITARY GLANDS OF INTACT FEMALE MICE BEARING A PITUITARY TRANSPLANT COMPARED TO PERCENTAGE OF ACIDOPHILES IN 0.5% TCA EXTRACTED PITUITARY GLANDS

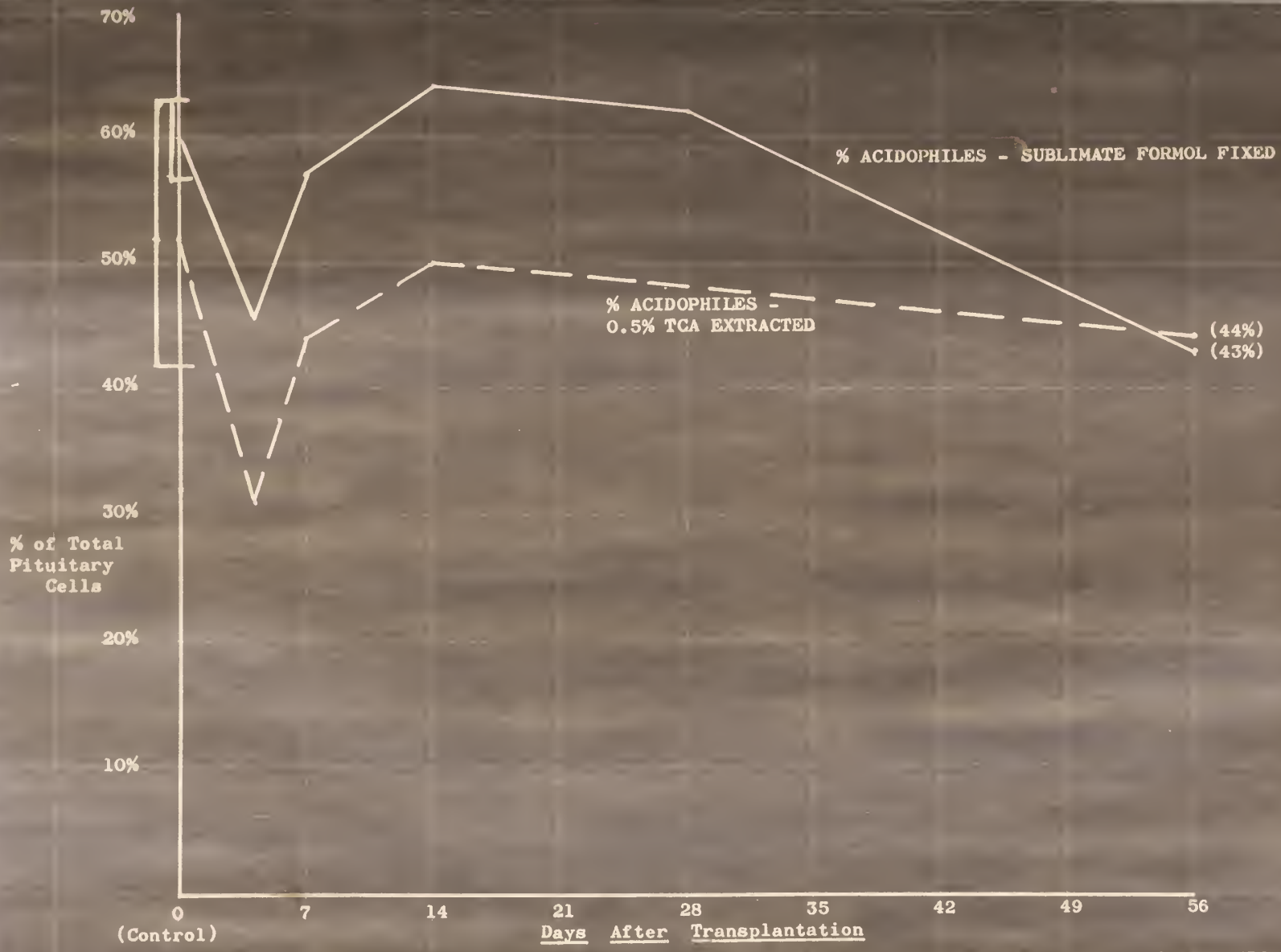


CHART 12 PERCENTAGE OF ACIDOPHILES IN THE SUBLIMATE FORMOL FIXED PITUITARY GLANDS OF MALE MICE BEARING A PITUITARY TRANSPLANT COMPARED TO PERCENTAGE OF ACIDOPHILES IN 0.5% TCA EXTRACTED PITUITARY GLANDS

DISCUSSION

Transplantation of the entire pituitary gland to sites remote from the hypothalamus has proved to be a valuable means for studying its function when removed from the humoral or neurogenic influence of the hypothalamus. Loeb and Kirtz (33) first reported that isologous transplants of pituitary glands in mice would not only persist, but induced intensified mammary growth and secretion in the host, and in some cases accelerated the carcinomatous transformation of the stimulated mammary glands. In spite of the fact that later work showed that the mammary tumor agent was responsible for most of these mammary tumors, their report that the transplants stimulated the glands and hastened the carcinomatous transformation implied that the graft was producing large quantities of a mammotrophic factor, ie., LTH. Everett (16, 17) extended this observation by showing that transplanted pituitary glands maintained the function of corpora lutea in rats to 4 or 5 times the duration of normal pregnancy and postulated that the transplantation removed the luteolytic mechanisms which operate in the intact rat and thus greatly prolonged corpora luteal life. This could only be explained on the basis of an excess production by the transplanted pituitary of LTH. Nikitovitch-Winer and Everett (35, 36, 37) also demonstrated that the transplanted pituitary graft, when re-transplanted under the median eminence of the hypothalamus, regained its normal gonadotrophic function and that estrous cycles returned

and some rats subsequently became pregnant. This furnished further evidence that the hypothalamic influence, presumably through the hypophyseal portal vessels, was necessary for production and secretion of the gonadotrophic hormones, except for LTH, which was apparently inhibited by the hypothalamus. Transplantation under the temporal lobe of the brain did not result in resumption of normal gonadotrophic function of the graft, showing the specificity of the hypothalamus.

Several reports of TSH, ACTH, and STH secretion in small amounts by the transplanted pituitary gland (25, 26, 29, 37) are not clear-cut, and emphasize that secretion is below levels normal for the intact pituitary. Muhlbock and Boot (34) showed that subcutaneous pituitary grafts in mice were associated with the production of LTH and induced mammary tumors in mice that were free ffom the mammary tumor agent. Gardner (18) has reported that pituitary tumors developed in pituitary glands transplanted subcutaneously into mice of several strains, and that the tumors or grafts were associated with well-developed mammary glands and lactation. He further demonstrated that when only one pituitary graft was present, the adjacent mammary glands usually showed more extensive lactation or more development and remote glands were not lactating. Abundant secretions accumulated in the mammary glands of mice bearing multiple pituitary grafts, even when the glands consisted almost entirely of ducts and were without appreciable alveoli.

The experiments reported here are largely in accord with the preceding series of observations on the function of the

transplanted pituitary grafts. These experiments were more concerned with the early changes induced by the transplanted pituitary grafts. Gardner's (18) observation that pituitary tumors appeared in the transplants which had been in place 500 days or more account for the absence of any tumorous development noted in these experiments. The other manifestations of function of the grafts, however, are seen at a much earlier date, but no investigators have quantitated these functions during the periods shortly after transplantation.

Vaginal smears taken in mice bearing pituitary transplants for periods from 4 days to 2 months support the finding that the graft produces LTH which influences the ovary to maintain the life of corpora lutea and extend the diestrous portion of the reproductive cycle to several times its usual length. Pseudopregnancy cycles were observed in the mice bearing pituitary transplants for short periods of time. Even at 4 days after transplantation, female mice with one or two transplants showed only 22% of days in which cornified cells could be identified in the vaginal smears compared with 45% in intact control females. At 30 days, cornified cells appeared on 11 percent of the days smeared. Histologic examination of ovaries also confirmed the presence of excess LTH in the animals bearing pituitary grafts. In female mice that had been hypophysectomized one day previous to the implantation of the pituitary graft, no ovarian function could be demonstrated. Vaginal smears showed no evidence of cornified cells and the weights of ovaries of hypophysectomized females dropped to less than one-fourth that of normal controls. This indicates

that the pituitary transplant in these mice was not producing gonadotrophic hormones sufficient to maintain the normal reproductive cycle. Similarly, hypophysectomized female mice bearing pituitary grafts failed to maintain uterine weight, even when the ovaries were left in situ. Male mice that had been hypophysectomized and had received one or two pituitary grafts showed a marked fall in testicular weight and in the weights of seminal vesicles and prostate glands, again indicating that the transplanted pituitary gland was not producing gonadotrophic hormones in sufficient quantities to maintain normal function.

Experiments in which hypophysectomized male and female mice received pituitary transplants and were weighed daily until autopsy indicated a subnormal production of STH by the transplanted pituitary gland. Hypophysectomy usually results in a small loss of body weight that is never regained, or at least body weight never increases beyond the weight at operation. The mice in this experiment lost one or two grams of body weight and after receiving a pituitary transplant one or two days later showed slight gains (one to three grams) of body weight for several days after transplant. However, by the end of 7, 14, or 30 days after transplantation, all mice had body weights that were less than the weight of the mice at the time of operation. This indicates that immediately after transplantation, the pituitary graft probably releases its supply of STH, that, once depleted, is not regained in appreciable amounts. If STH is produced by the pituitary

grafts, it is at a subnormal rate.

A careful study was made in these experiments of the early effects of the pituitary transplant on the mammary glands. Gardner (18) and Muhlbock (34) reported that the mammary glands showed evidence of excessive stimulation after the grafts had been in place for many months, but no investigator has examined mammary glands at varying intervals of time immediately after transplantation, or has reported the effect of castration or hypophysectomy on mammary gland development in mice with transplants. In the experiments reported here, the presence of even one pituitary graft quickly and profoundly affected the mammary glands. Four days after transplantation, intact female mice bearing one pituitary graft on the right side showed a marked alveolar stimulation of those mammary glands immediately around the graft, while those at a distance from the graft were much less developed. If a pituitary graft resided in or very near a mammary gland, local proliferation of the alveoli was noted, and mammary glands at a distance from the graft or on the side oppostie the graft showed less stimulation. After 1 month, intact female mice bearing one or two grafts showed intense mammary stimulation with milk or milk-like substance in the ducts. In some instances milk was seen in the ducts of the mammary gland that was nearest to the graft, while more distant glands did not have milk-filled ducts. By 2 months after transplantation, all ducts were milk-filled, regardless of the placement or number of grafts.

Male mice bearing pituitary transplants never showed mammary development, indicating the need for ovarian hormones

for the development of the mammary glands. Castrate and hypophysectomized mice with pituitary grafts showed no development of the mammary glands. In hypophysectomized mice, extreme atrophy of the mammary glands resulted, despite the pituitary transplants.

In summary then, the pituitary grafts in the subcutaneous tissues of the mouse elaborated excessive quantities of LTH and diminished quantities of other pituitary hormones. Removal of the pituitary gland from the proximity of the hypothalamus resulted in inhibition of the secretion of other pituitary hormones and an increased secretion of LTH. The excessive LTH produced by the graft acted on the ovary to maintain prolonged function of the corpora lutea. The dual action of LTH on the corpora lutea and on the mammary glands induced mammary development similar to that seen in pregnancy. The influence of FSH and LH on the ovary was partially blocked and the estrous cycle was prolonged in the diestrous phase.

Several investigators have studied the histological and histochemical characteristics of transplanted pituitary glands in an effort to identify the pituitary cells that persist and to determine hormone content and function of the graft cells. Nikitovitch-Winer and Everett (38) studied the histologic changes of rats' pituitary glands grafted on the kidney and upon re-transplantation under the median eminence using the PAS and aldehyde-fuchsin techniques. Their observations were confined principally to the gonadotrophs and thyrotrophs; no attempt was made to evaluate acidophiles.

Gonadotrophs persisted in the kidney graft for 2 to 3 weeks and thyrotrophs for one week, after which these cells were rarely recognized.

Siperstain and Greer (56) used the PAS and Mallory stain to study the histocytology of the mouse's pituitary gland after transplantation to the anterior eye chamber. They used pituitary glands of new-born mice as donors and studied the appearance of these grafts from 1 day to 14 months. Acidophiles decreased in number by the 15th day and were very rare by the 51st day. No definite basophiles were identified after 11 days. The chromophobes were larger in the transplants than those in intact pituitary glands and had cytologic manifestations of activity, principally increased cytoplasmic basophilia. The size of the intermediate lobe in the transplanted pituitaries increased.

No investigators have studied the transplanted pituitary gland placed in the subcutaneous space of the mouse in reference to its histochemical appearance at varying short intervals of time or its effect on the pituitary gland of the host. Siperstein and Greer (56) used the anterior chamber of the mouse's eye as the site of transplantation, but did not correlate the microscopic appearance of the graft with the hormonal effects of the graft on the other endocrine glands and reproductive organs of the animals or did not investigate the graft's influence on the host's pituitary gland. Nor did they investigate the histologic characteristics of the transplant in castrate, hypophysectomized, or estrogen-injected mice,

except to state that "a preliminary experiment revealed no apparent morphologic differences between implants in animals which were untreated, hypophysectomized, hypophysectomized and castrated, and hypophysectomized and fed propylthiouracil." They present no data from this experiment. Other investigators (29, 30, 38, 62) who have studied the cytology of anterior lobe transplants in animals of different strains have confined their principal observations to the fate of the basophiles, while largely ignoring the acidophiles, presumably because they did not have an effective stain for them. This is surprising considering the evidence which has accumulated that the cells which produce LTH in the pituitary gland are the acidophiles (8, 12, 28, 52). And since the transplanted pituitary gland produces such excessive quantities of LTH, the investigation of the acidophiles in the transplants seems of great importance.

In the experiments reported here, the general characteristics of transplanted pituitary glands are similar to those that others have observed (29, 30, 38, 56, 62). Transplantation resulted in a massive central infarct of the pituitary graft that was surrounded by a 3-4 cell leyer of normal-appearing pituitary cells. A very early vascular response was elicited in the subcutaneous space, so that by even 4 days, capillaries invaded the graft on both a gross and microscopic level. Mitoses appeared in the anterior lobe cells during the first week, but were not seen thereafter. At the end of one month, resolution and scarring of the central necrotic area were complete, inflammatory response had subsided and vascularization

was extensive and complete. No remnant of the neural lobe could be identified in the grafts. Intermediate lobe tissue, as reported also by Siperstein and Greer (56) and by Knigge (29), underwent hyperplasia when transplanted, so that in some instances, the volume of intermediate lobe tissue in the graft exceeded the volume of anterior lobe tissue. Thus it appears that interference with the normal hypothalamic-pituitary relationship also results in hypertrophy of intermediate lobe tissue. Whether increased function and secretion of intermediate lobe tissue resulted from transplantation was not evaluated.

Luxol Fast Blue was an excellent stain for acidophiles in the mouse's pituitary gland and in the pituitary transplants. The intense blue color of the acidophiles contrasted sharply with the red of the basophiles (PAS reaction), and the color of the acidophiles retained the intensity of the stain throughout the staining procedure, once the initial differentiation had been made. Orange G, the other frequently used acidophilic stain, was much more unreliable in staining properties, tending to wash out easily when the tissue was dehydrated in alcohols and giving a very light stain to the cells. Aldehyde-fuchsin was a very difficult and unreliable stain for thyrotrophs, so it was not used in an attempt to differentiate thyrotrophs from gonadotrophs in the pituitary transplants. This differentiation was made on morphologic grounds and on the location of the cells in "zones" in the intact pituitary gland.

Extraction of pituitary glands and grafts in 2.5% TCA

as described by Barrnett et al. (5) to localize the cells that produce LH did not prove to be a valuable technique. Since this technique was described for use with rat pituitary glands, no assumption that it localized LH in the mouse could be validly made. Because the primary object of the study was not in LH, and because in most cases 2.5% TCA allowed the same staining of basophiles with PAS as did sublimate-formol fixation alone, results with the technique were not described.

Extraction of pituitary glands and grafts in 0.5% TCA as described by Barrnett et al. (8) to localize the cells that produce LTH also proved to be non-rewarding. This technique was also described for use in rats' pituitary glands, and therefore one cannot apply it with complete validity to the mouse, because mouse LTH may differ chemically from rat LTH. In spite of this, however, comparisons of pituitary glands and grafts extracted in 0.5% TCA with those fixed directly in sublimate-formol were made. These indicated that there was no demonstrable difference between the numbers of acidophiles that stained with LFB in 0.5% TCA extracted pituitaries and grafts and in routinely fixed specimens. Thus 0.5% TCA did not differentiate reliably between acidophiles containing LTH and those containing other acidophilic hormones, either in the intact pituitary gland or the pituitary graft. Even in the pregnant and post-partum pituitary gland of the female mouse, no increased numbers of acidophiles from 0.5% TCA extracted pituitaries could be demonstrated.

Siperstein and Greer (56) reported observing PAS-positive

basophiles in pituitary grafts up to the 11th day of transplantation, after which only rare, questionable, PAS cells were identified. Everett (38) reported the absence of basophiles after 2 to 3 weeks. In the experiments reported here PAS-positive basophiles were identified in moderate numbers at 14 days after transplant, and only a few basophiles persisted after this time. The basophiles were much smaller by 7 days after transplantation and had sparse, coarsely clumped PAS cytoplasmic granules after 14 days.

Siperstein and Greer (56) reported that acidophiles were less frequent in pituitary transplants than in the pituitary gland, and appeared less frequently with time until they were very rare by the 51st day. This investigation found that acidophiles at 14 days were numerous and stained with a moderate intensity in intact mice with transplants, although the size of the acidophiles had decreased to about 2/3 of their former size. Moderate to numerous numbers of acidophiles were seen in the grafts at the end of one month and also at two months after transplantation. Some stained intensely and uniformly with LFB but others had a foamy-appearing LFB material in the cytoplasm, and the cells were slightly larger in many cases than the more intensely staining acidophiles. The acidophiles remained about 2/3 normal size, even at 2 months.

Siperstein and Greer (56) reported that chromophobes increased in size in their transplants, associated with increasing numbers of Golgi images and increased size of

nucleoli. Cytoplasmic basophilia increased in these chromophobes, resulting from increased accumulation of RNA, and they speculated that these chromophobes may be the active secretory elements of the transplants. The chromophobes in the present study had not increased in size appreciably and an increased number of Golgi images were not observed. Cytoplasmic basophilia was not evaluated because the appropriate stains were not used. These "chromophobes" reported by Siperstein need not be the site of production of LTH, however, because they were not observed in that investigator's transplants until approximately the 20th day, which as has been shown, is much later than the excessive production of LTH by the graft, observed as early as four days after transplantation. Also, acidophiles persist in the grafts to at least 2 months after transplantation in good numbers.

The present study confirmed the observation by Siperstein (56) and Knigge (29) that the pars intermedia increases in size in the pituitary transplants, and that by the 14th day after transplantation, many of the intermedia cells stain intensely with PAS while other, somewhat larger intermedia cells stain lightly with PAS.

Pituitary grafts from male and female mice that had been castrated and transplanted 1 month previously showed very few, sparsely granulated basophiles and numerous, intensely stained acidophiles. They resembled the grafts in normal animals at the same time after transplantation. Similarly, grafts from animals that had been hypophysectomized and

transplanted up to one month previously did not differ from the grafts of normal animals. Thus these two procedures did not seem to influence the cytology of the graft.

Acidophiles in transplants in estrogen-treated mice were numerous and appeared somewhat larger than the acidophiles of intact untreated mouses' pituitary transplants. Grafts in estrogen-treated mice had small acidophiles with intensely stained LFB material, and large cells with a foamy LFB material in the cytoplasm. No particular significance could be attached to this finding.

Only one investigation of the histologic evaluation of the pituitary glands of host mice bearing pituitary transplants has been made. Wolfe, Kirtz, and Loeb (62) made cell counts in some of the anterior pituitary glands of the host mice bearing pituitary transplants and suggested that basophiles were more abundant in the glands of ovariectomized host mice than in glands of non-ovariectomized animals, and these were generally degranulated. Castration cells were not observed. Pituitary glands from male and female control mice stained with PAS and LFB had fewer acidophiles and basophiles in the female pituitary glands than did the pituitary glands of male mice. The decreased number of basophiles in pituitary glands of female mice after 40 to 50 days of age has been reported by Yamada (63) and by Siperstein *et al.* (55) in the rat, and has been considered as due to the release of gonadotrophic hormones coinciding with the first estrus. The lower number of acidophiles in pituitary glands of females

has not been reported.

The intact pituitary glands of both male and female mice bearing pituitary grafts and of mice without pituitary grafts showed no differences in the numbers of basophiles and acidophiles. Thus the pituitary graft did not modify the cytology of the host's pituitary gland. In estrogen-treated mice bearing pituitary grafts, however, the number of basophiles in the pituitary gland of the host was markedly reduced. The basophiles appeared degranulated and stained very lightly with PAS. This effect of estrogen treatment has been reported before (61). Injections of estrogen into the rat induced degranulation of the basophiles, accompanied by increased prominence of the mitochondria and hypertrophy of the Golgi apparatus. Thus the reduction in number of basophiles in this experiment was due to degranulation of the basophiles, making them difficult to identify with the PAS reaction, and was probably not due to the effect of the pituitary graft.

SUMMARY

Intact pituitary glands were transplanted into the subcutaneous space of isologous hosts of the PC hybrid group of mice. The mice were autopsied from 12 hours to 2 months after transplantation, and the effects of the grafts on the hosts were noted. The grafts were studied histochemically, by means of a Periodic Acid Schiff--Luxol Fast Blue stain, and the general microscopic characteristics and relative numbers of pituitary cells were noted.

Most of the pituitary grafts to the subcutaneous tissues of male and female mice were found at autopsy. No enlargements or tumors were found in any of the transplanted glands. Vaginal smears taken in mice bearing pituitary transplants showed pseudopregnancy cycles, indicating that the graft produced LTH that maintained the function of corpora lutea for prolonged periods. Histologic examination of the ovaries showed increased size and activity of the corpora lutea. The weights of the uteri, testes, seminal vesicles and prostate, and submaxillary glands of intact mice bearing pituitary grafts were not different from those of intact mice without grafts, but these organs were not maintained by the pituitary graft in hypophysectomized mice.

The presence of one or two pituitary grafts quickly and profoundly affected the mammary glands. As early as four days after transplantation, proliferation of the alveoli and ducts was observed, more marked in the areas of mammary gland directly adjacent to the graft. By two months after trans-

plantation, all mammary glands were well-developed and lactating. These findings also indicate that the pituitary grafts elaborated excessive quantities of LTH when removed from the inhibiting influence of the hypothalamus.

Microscopically, transplantation of the pituitary gland resulted in a massive central infarct of the gland surrounded by a 3-4 cell layer of normal-appearing pituitary cells. By 14 days after transplantation, this area had resolved and scarred and the anterior lobe tissue had proliferated. Intermediate lobe tissue proliferated extensively, but no remnant of the neural lobe was identified.

PAS-positive basophiles decreased in the transplanted gland, until only a few lightly stained basophiles could be identified 1 month after transplantation. LFB-positive acidophiles were numerous and intensely stained, even after two months of transplantation. The sizes of both basophiles and acidophiles decreased markedly after transplantation. Pituitary grafts in hosts that had been castrated, hypophysectomized, or estrogen-treated did not differ markedly from those in intact hosts.

Histologic evaluation and cell counts were performed on the pituitary glands of mice bearing pituitary grafts subcutaneously to evaluate the effect of the graft on the cytology of the pituitary gland. The intact pituitary glands of both male and female mice bearing pituitary grafts and of mice without pituitary grafts showed no significant differences in the cytology or in the numbers of basophiles and acidophiles.

REFERENCES

1. Adams, C.W. M. and Swettenham, K.V. 1958
The Histochemical Identification of Two Types of Basophil Cell in the Normal Human Adenohypophysis.
J. Path. and Bact. 75: 95.

2. Barrnett, R. and Seligman, A.M. 1952
Histochemical Demonstration of Protein-Bound Sulfhydryl Groups.
Science 116: 323.

3. Barrnett, R. and Seligman, A.M. 1954
Histochemical Demonstration of Sulfhydryl and Disulfide Groups of Protein.
J. Nat. Cancer Inst. 14: 769.

4. Barrnett, R., Ladman, A.J., and McAllaster, N.J. 1955
An Abstract on Localization of Glycoprotein Hormones In the Adenohypophysis.
J. Histochem. and Cytochem. 3: 391.

5. Barrnett, R., Ladman, A.J., McAllaster, N.J., and Siperstein, E.R. 1956
The Localization of Glycoprotein Hormones in the Anterior Pituitary Glands of Rats Investigated by Differential Protein Solubilities, Histological Stains and Bio-assays.
Endocrinology 59: 398.

6. Barrnett, R., Siperstein, E.R., and Josimovich. 1956
The Localization of Simple Protein Hormones in the Adenohypophysis.
Anat. Rec. 124: 388.

7. Barrnett, R.J. and Seligman, A.M. 1958
Histochemical Demonstration of Protein-Bound Alpha-Acylamido Carboxyl Groups.
J. Biophysical and Biochemical Cytology 4: 169.

8. Barrnett, R.J., Roth, W.D., and Salzer, J. 1961
The Histochemical Demonstration of the Sites of Luteotropic Hormone in the Rat Pituitary Gland.
Endocrin. 69: 1047.

9. Catchpole, H.R. 1949
Distribution of Glycoprotein Hormones in the Anterior Pituitary Gland of the Rat.
J. Endocrinology 6: 218.

10. Cushing, H., and Davidoff, L.M. 1927
The Pathological Findings in Four Autopsied Cases of Acromegaly With a Discussion of Their Significance.
Rockefeller Inst. Med. Res. Monogr. 22: 1.

11. Dawson, A.B. 1939
Differential Staining of the Anterior Pituitary Gland of the Cat.
Stain Tech. 14: 133.

12. Dawson, A.B. 1946
Some Evidences of Specific Secretory Activity of the Anterior Pituitary Gland of the Cat.
Amer. J. Anatomy 78: 347.

13. Dawson, A.B. 1954
The Demonstration By Differential Staining of Two Types of Acidophils in the Anterior Pituitary Gland of the Rat.
Anat. Record 120: 810.

14. Dawson, A.B. and Friedgood, H.B. 1938
Differentiation of Two Classes of Acidophils in the Anterior Pituitary of the Female Rabbit and Cat.
Stain Tech. 13: 17.

15. Elftman, H. and Wegelius, O. 1959
Anterior Pituitary Cytology of the Dwarf Mouse.
Anat. Rec. 135: 43.

16. Everett, J.W. 1954
Luteotrophic Function of Autografts of the Rat Hypophysis.
Endocrin. 54: 685.

17. Everett, J.W. 1956
Functional Corpora Lutea Maintained for Months by Autografts of Rat Hypophyses.
Endocrin. 58: 786.

18. Gardner, W.U. 1962
Some Studies on Experimental Tumorigenesis: Tumors in Transplanted Pituitary Glands.
From On Cancer and Hormones, Univ. Chicago, p. 89.

19. Halmi, N.S. 1950
Two Types of Basophils in the Anterior Pituitary of the Rat and Their Respective Cytophysiological Significance.
Endocrin. 47: 289.

20. Halmi, N.S. 1952
Differentiation of Two Types of Basophiles in the Adenohypophysis of Rat and Mouse.
Stain. Tech. 27: 61.

21. Halmi, N.S. 1952
Two Types of Basophils in the Rat Pituitary: "Thyrotrophs" and "Gonadotrophs" vs. Beta and Delta Cells.
Endocrin. 50: 140.

22. Halmi, N.S. and DeGroote. 1961
Remarks Concerning Two Types of Basophil Cells in the Human Adenohypophysis.
J. Clin. Endocrin. 21: 732.

23. Haterius, H.O., Schweizer, M., and Charipper, H. 1935
Experimental Studies of the Anterior Pituitary. III. Observations on the Persistence of Hypophyseal Transplants in the Anterior Eye Chamber.
Endocrin. 19: 673.

24. Hellbaum, A.A., McArthur, L.G., Campbell, P.J. and Finerty, J.C. 1961
The Physiological Fractionation of Pituitary Gonadotropic Factors Correlated With Cytological Changes.
Endocrin. 68: 144.

25. Hertz, R. 1959
Growth in the Hypophysectomized Rat Sustained by Pituitary Grafts.
Endocrin. 65: 926.

26. Hertz, R. 1960
Gonadotropin and Adrenocorticotropin From Rat Pituitary Homografts As Manifested by Host Response to Chorionic Gonadotropin and Amphenone.
Endocrin. 66: 842.

27. Hildebrand, LE, Rennels, E.G., and Finerty, J.C. 1957
Gonadotrophic Cells of the Rat Hypophysis and Their Relation to Hormone Production.
Ztschr. Zellforsch. 46: 400.

28. Hymer, W.C., McShan, W.H., and Christiansen, R.G. 1961
Electron Microscopic Studies of Anterior Pituitary Glands from Lactating and Estrogen-Treated Rats.
Endocrin. 69: 81.

29. Knigge, K.M. 1961
Pituitary Ocular Graft Function in the Rat, With a Comparison of the Qualitative Nature of TSH From Normal Pituitaries and Ocular Grafts.
Endocrin. 68: 101.

30. Kovacs, K. 1961
Histological Alterations in the Rat Pituitary Transplanted to the Eye.
J. Endocrin. 23: 109.

31. Ladman, A.J. and Barrnett, R.J. 1956
Variations of the Histochemically Demonstrable Protein-Bound Sulfhydryl and Disulfide Groups in Cells of the Anterior Pituitary Gland under Various Endocrine Conditions.
J. Morph. 98: 305.

32. Leznoff, A., Fishman, J., Goodfriend, L., McGarry, El, Beck, J., Rose, B. 1960
Localization of Fluorescent Antibodies to Human Growth Hormone in Human Anterior Pituitary Glands.
Proc. Soc. Exp. Biol. (N.Y.) 104: 232.

33. Loeb, L. and Kirtz, M.M. 1939
The Effects of Transplants of Anterior Lobes of the Hypophysis on the Growth of the Mammary Gland and on the Development of Mammary Gland Carcinoma in Various Strains of Mice.
Amer. J. Cancer 36: 56.

34. Muhlbock, O., and Boot, LM. 1959
Induction of Mammary Cancer in Mice Without the Mammary Tumor Agent by Isografts of Hypophyses.
Cancer Research 19: 402.

35. Nikitovitch-Winer, M.B. and Everett, J.W. 1957
Resumption of Gonadotrophic Function in Pituitary Grafts Following Retransplantation From Kidney to Median Eminence.
Nature 180: 1434.

36. Nikitovitch-Winer, M.B. and Everett, J.W. 1958
Comparative Study of Luteotrophin Secretion By Hypophyseal Autotransplants in the Rat.
Endocrin. 62: 522.

37. Nikitovitch-Winer, M.B. and Everett, J.W. 1958
Functional Restitution of Pituitary Grafts Re-transplanted From Kidney to Median Eminence.
Endocrin. 63: 916.

38. Nikitovitch-Winer, M.B. and Everett, J.W. 1959
Histocytologic Changes in Grafts of Rat Pituitary in the Kidney and Upon Retransplantation Under the Diencephalon.
Endocrin. 65: 357.

39. Paget, G.E. and Eccleston, E. 1959
Aldehyde Thionine--A Stain Having Similar Properties to Aldehyde Fuchsin.
Stain. Tech. 34: 223.

40. Paget, G.E. and Eccleston, E. 1960
Simultaneous Specific Demonstration of Thyrotroph, Gonadotroph and Acidophil Cells in the Anterior Hypophysis.
Stain. Tech. 35: 119.

41. Pearse, A.G.E. 1949
The Cytochemical Demonstration of Gonadotropic Hormone in the Human Anterior Hypophysis.
J. Path. and Bact. 61: 195.

42. Pearse, A.G.E. 1950
Differential Stain for Human and Animal Anterior Hypophysis.
Stain. Tech. 25: 95.

43. Pearse, A.G.E. 1952
Observations on the Localization, Nature, and Chemical Constitution of Some Components of the Anterior Hypophysis.
J. Path. and Bact. 64: 791.

44. Pearse, A.G.E. 1952
The Cytochemistry and Cytology of the Normal Anterior Hypophysis Investigated by the Trichrome-Periodic Acid Schiff Methods.
J. Path. and Bact. 64: 811.

45. Perry and Lochhead 1939
Histological Technique for Pituitary Gland of the Mouse.
Bull: Int. Assn. of Med. Museums. 19: 101.

46. Purves, H.D. 1961
Hypophyseal Morphology.
In Sex and Internal Secretions, 3rd. ed., Young, W.C., Ed. p. 161, Williams and Wilkins Co., Baltimore.

47. Purves, H.D. and Griesbach, W.E. 1951
The Site of Thyrotropin and Gonadotropin Production in the Rat Pituitary Studied by McManus-Hotchkiss Staining For Glycogen.
Endocrin. 49: 244.

48. Purves, H.D. and Griesbach, W.E. 1951
Specific Staining of the Thyrotrophic Cells of the Rat Pituitary by the Gomori Stain.
Endocrin. 49: 427.

49. Purves, H.D. and Griesbach, W.E. 1954
The Site of Follicle Stimulating and Luteinizing Hormone Production in the Rat Pituitary.
Endocrin. 55: 785.

50. Rennels, E.G. 1957
Two Tinctorial Types of Gonadotrophic Cells in the Rat Hypophysis.
Ztschr. Zellforsch. 45: 464.

51. Rennels, E.G. 1962
An Electron Microscope Study of Pituitary Autograft Cells in the Rat.
Endocrin. 71: 713.

52. Sanders, A.E. and Rennels, E.G. 1959
Evidence on the Cellular Source of Luteotropin Derived From a Study of Rat Pituitary Autografts.
Ztschr. Zellforsch. 49: 263.

53. Severinghaus, A.E. 1939
Anterior Hypophyseal Cytology in Relation to the Reproductive Hormones.
In Sex and Internal Secretions, 2nd. ed., Allen, Danforth and Doisey, Eds., p. 1045. Williams and Wilkins Company, Baltimore.

54. Shanklin, W.M., Nassar, T.K., and Issidorides, M. 1959
Luxol Fast Blue as a Selective Stain for Alpha Cells in the Human Pituitary.
Stain. Tech. 34: 55.

55. Siperstein, E., Nichols, G., Griesbach, W. and Charkoff, I. 1954
Cytological Changes in the Rat Anterior Pituitary Gland from Birth to Maturity.
Anat. Rec. 118: 593.

56. Siperstein, E.R. and Greer, M.A. 1956
Observations on the Morphology and Histochemistry of the Mouse Pituitary Implanted in the Anterior Eye Chamber.
J. Nat. Canc. Inst. 17: 569.

57. Smith, P. 1961
Postponed Homotransplants of the Hypophysis into the Region of the Median Eminence in Hypophysectomized Male Rats.
Endocrin. 68: 130.

58. Smith, P.E. and Mac Dowell, E.C. 1930
An hereditary Anterior Pituitary Deficiency in the Mouse.
Anat. Rec. 46: 249.

59. Thomas, F. 1938
Technic for Hypophysectomy of the Mouse.
Endocrin. 23: 99.

60. Wilson, W.D. and Ezrin, C. 1954
Three Types of Chromophil Cells of the Adenohypophysis.
Amer. J. Path. 30: 891.

61. Wolfe, J.M. 1949
Cytochemical Studies of the Anterior Hypophyses of Rats receiving Estrogen.
Amer. J. Anat. 85: 309.

62. Wolfe, J.M., Kirtz, M.M., and Loeb, L. 1940
The Cellular Constitution of Transplants of the Anterior Hypophysis of Inbred Strains of Mice.
Amer. J. Cancer 38: 239.

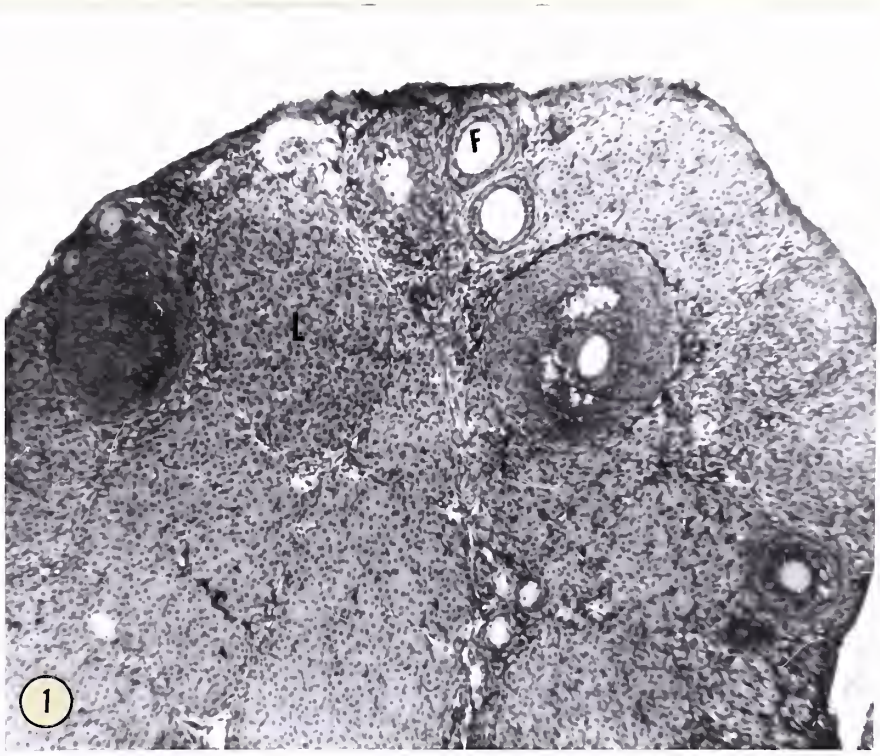
63. Yamada, K., Sano, M., and Ito, T. 1957
A Postnatal Histogenetic Study of the Anterior Pituitary of the Mouse.
Folia. Anat. Jap. 30: 177.

PHOTOMICROGRAPHS

PHOTOMICROGRAPHS

FIGURE 1. Section of an ovary from a normal control mouse with no pituitary transplant. Approximately three generations of corpora lutea (L) are seen. Follicles (F) in different stages of development are present. H. & E. Stain. 93x.

FIGURE 2. Section of an ovary from a mouse that bore a pituitary transplant in the subcutaneous space for 1 month. The corpora lutea (L) are increased in size over those of the control ovary and corpora lutea cells are larger than control luteal cells. Approximately two generations of corpora lutea are recognized and their total number is increased over that of the control overy. A few small antral follicles (F) are seen in the ovary of the transplant-bearing mouse. H. & E. Stain. 93x.


F
L
1

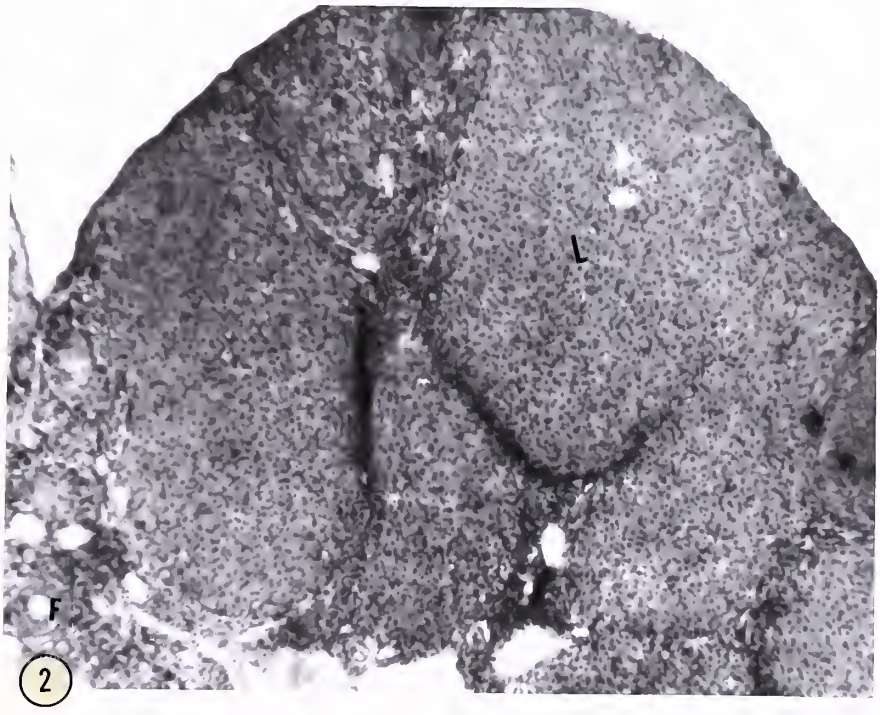

L
F
2

FIGURE 3. Mammary gland (3rd. gland on left side) from female mouse with no pituitary transplant. This gland shows minimal alveolar and ductal development and the stage of development rates 1 on a scale from 0 to 4. 4.6x.

FIGURE 4. Mammary gland (2nd. gland on right side) from female mouse with a pituitary gland transplanted subcutaneously for 4 days on the right side. This gland shows a marked increase in alveolar and ductal development. The stage of development was rated at 2. 4.6x.


3


4

FIGURE 5. Mammary gland (3rd. gland on right) from female mouse with pituitary glands transplanted subcutaneously for 7 days on both the right and left sides. Alveolar and ductal development are pronounced. The pituitary graft was removed from the oval area at the right of the mammary gland, and a local intense stimulation of the gland is seen in the area where the graft resided. Immediately around the graft the mammary gland shows a stage of development rated at 3, while at the periphery of the gland, the stage of development is rated at 1+. 4.6x.

FIGURE 6. Mammary gland (3rd. gland on right) from a female mouse with one pituitary gland transplanted for 14 days on each side. The marked alveolar and ductal development is rated at 3 with areas of 2+. 4.6x.


5


6

FIGURE 7. Mammary gland (3rd. gland on right) from female mouse with one pituitary gland transplanted for 30 days on each side. Intense alveolar and ductal development, rated at 3 with areas of 2+, are noted. Milk or milk-like substance was seen in the ducts at autopsy. 4.6x.

FIGURE 8. Mammary gland (2nd. gland on right) from an ovariectomized mouse with a pituitary transplant for 30 days on the right side. The mammary gland is atrophic with little or no alveolar development. This gland rated 1-. 4.6x


7

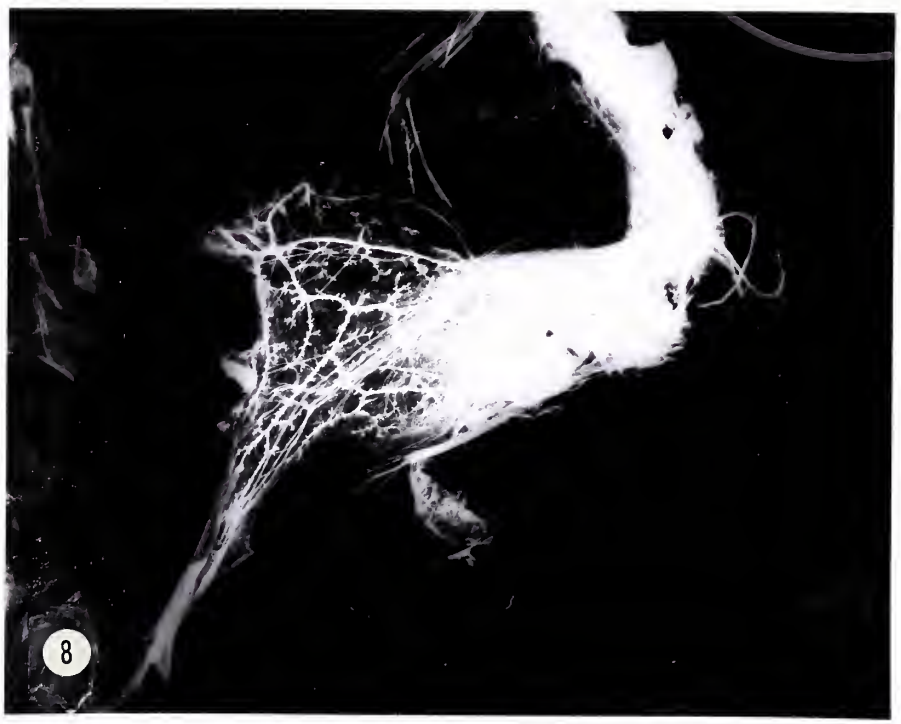
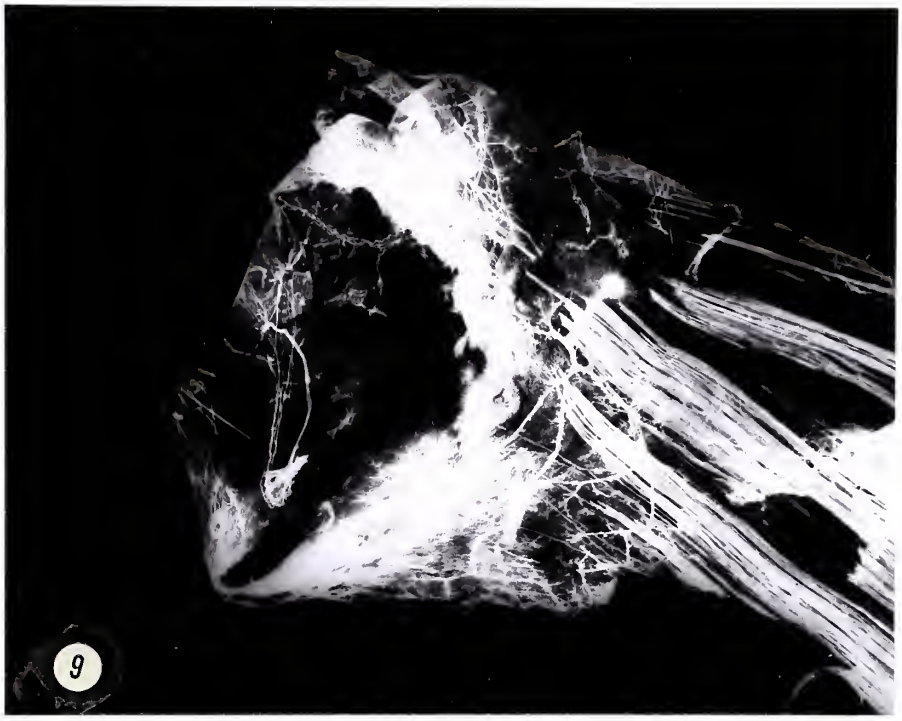

8

FIGURE 9. Mammary gland (3rd. gland on right) from a hypophysectomized female mouse with a pituitary gland transplanted for 30 days on the right side. Extreme atrophy of the mammary gland, rated at 0, indicates that the graft had no mammatrophic effect. 4.6x

FIGURE 10. Mammary gland (3rd. gland on right) from a female mouse that had a pituitary gland transplanted for 30 days and that received estrogen injections for the same period. Intense stimulation of the gland is noted, rated at 2+. 4.6x


9

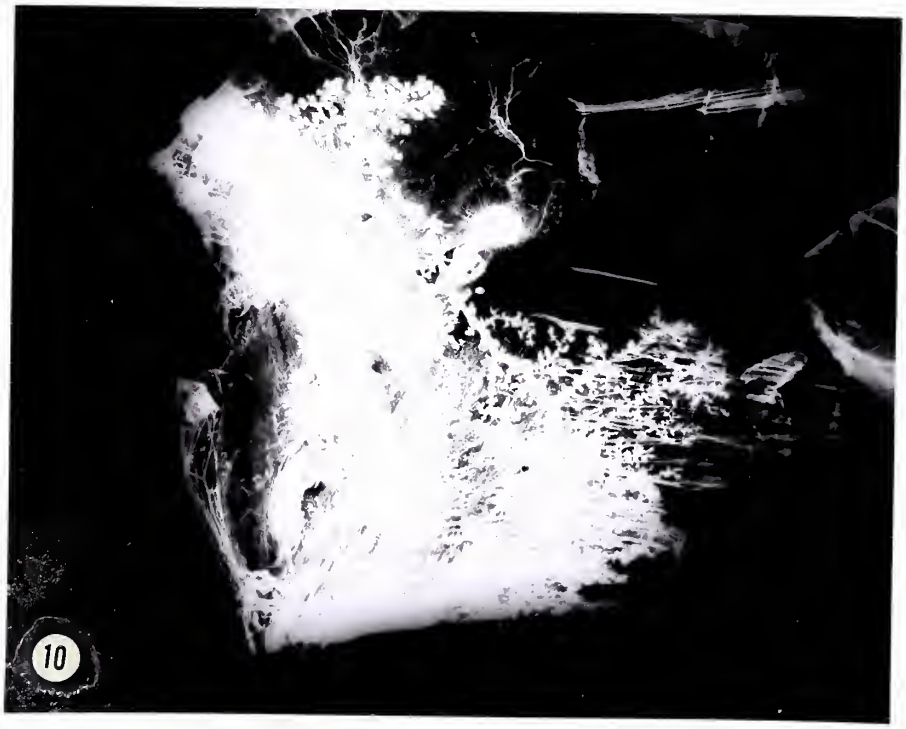

10

FIGURE 11. Mammary gland (2nd. gland on right) from a female mouse with a pituitary gland transplanted for 60 days on the right side. Extensive mammary development, rated at 2+ with areas of 3, and milk-filled ducts are noted. 4.6x

FIGURE 12. Section from subcutaneous pituitary graft 24 hours after transplantation. A massive central infarct (I) occupies the central portion of the graft with degenerating cells and a heavy inflammatory response. H. & E. Stain. 93x


11

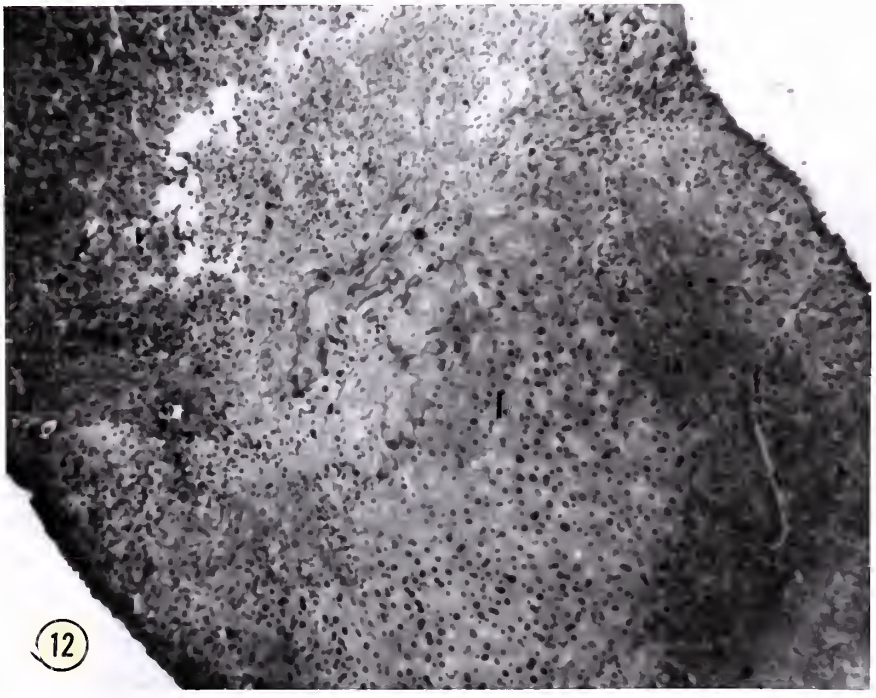
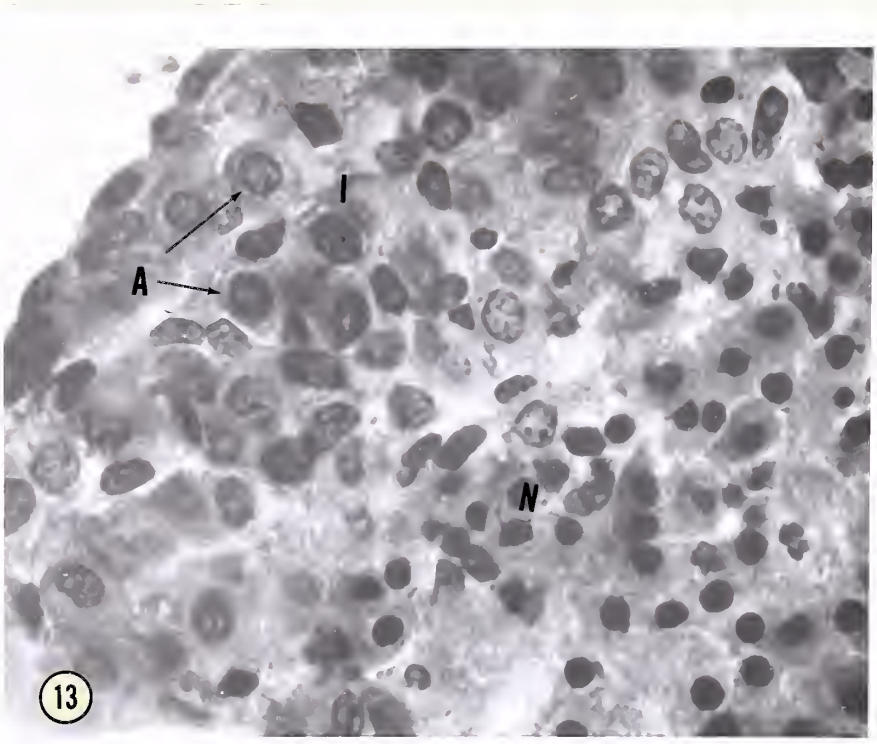
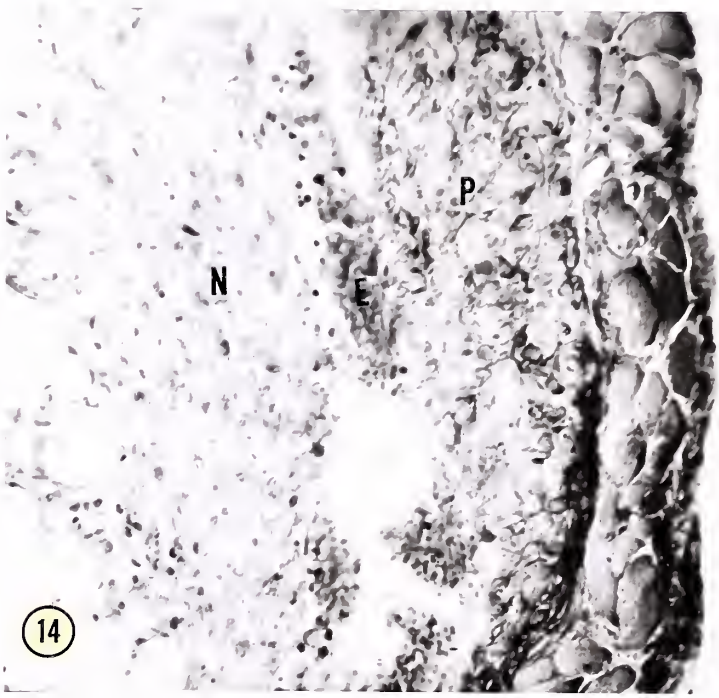

12

FIGURE 13. Higher power view of Figure 12 showing the layer of 3-4 normal-appearing pituitary cells at the outer margin of the graft. This layer of intact cells (I) contains acidophiles (A) that are normal in appearance. Toward the central regions of the graft, nuclei (N) are pyknotic and cell borders are indistinct. H. & E. Stain. 880x

FIGURE 14. Section from subcutaneous pituitary graft 4 days after transplantation. A layer of normal-appearing pituitary cells (P) surround an area of necrosis (N) and engorgement with erythrocytes (E). PAS-Luxol Fast Blue Stain. 235x



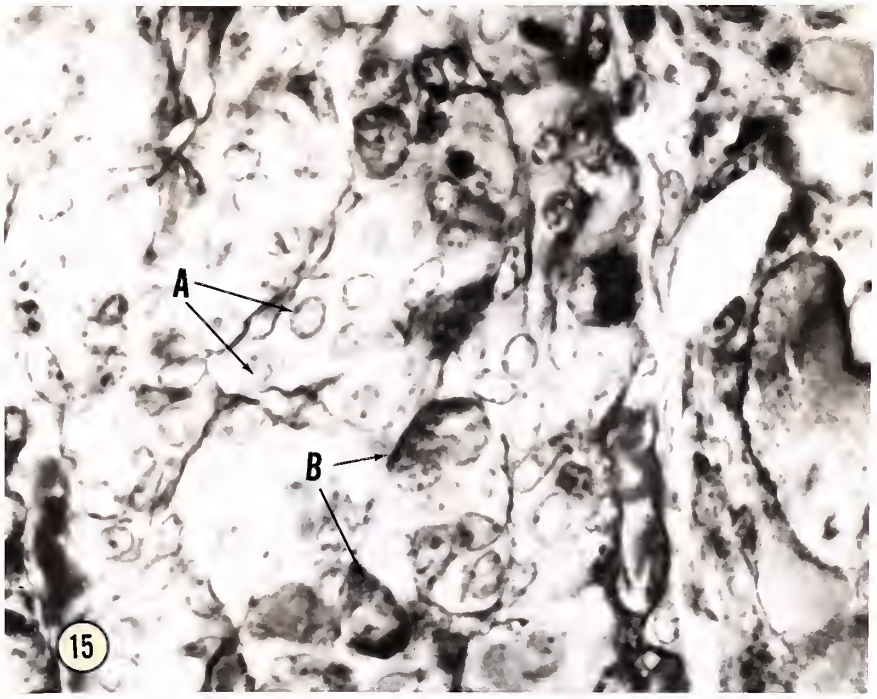
13



14

FIGURE 15. Same graft as Figure 14 showing numerous basophiles (B) staining with PAS and numerous Acidophiles (A) staining with LFB. Vascular spaces are evident in the section. 880x

FIGURE 16. Same graft as Figure 14. Round cells with clear cytoplasm are acidophiles (A) and the angular cells with coarse clumped PAS material are basophiles (B). 2275x


A
B
15

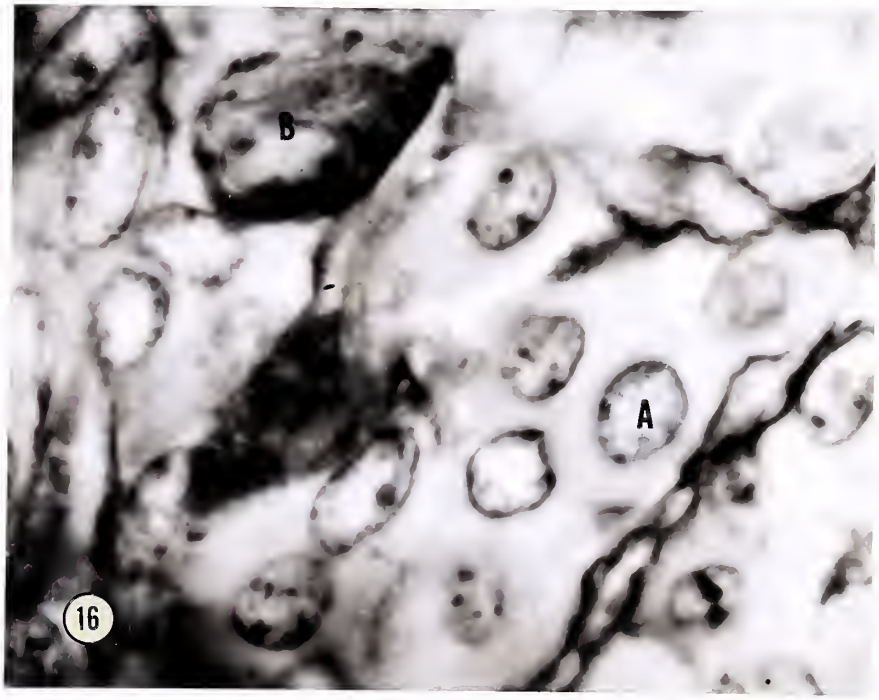
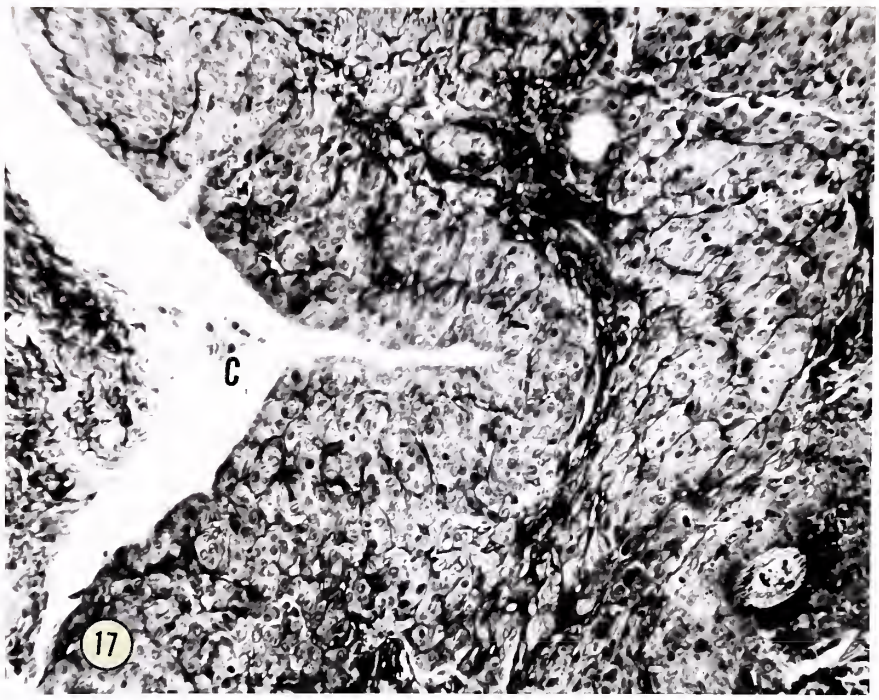

B
A
16

FIGURE 17. Section from subcutaneous graft of a pituitary gland 7 days after transplantation. An intraglandular cleft (C) is evident. The layer of normal pituitary cells occupies the area to the right of the cleft and contains many basophiles and acidophiles. The inflammatory response is diminished and fibroblastic proliferation invades the necrotic portions of the graft. PAS-LFB Stain. 235x

FIGURE 18. Same graft as Figure 17 showing intraglandular cleft (C) and many moderately stained basophiles (B) and numerous intensely stained acidophiles (A). 880x


C
17

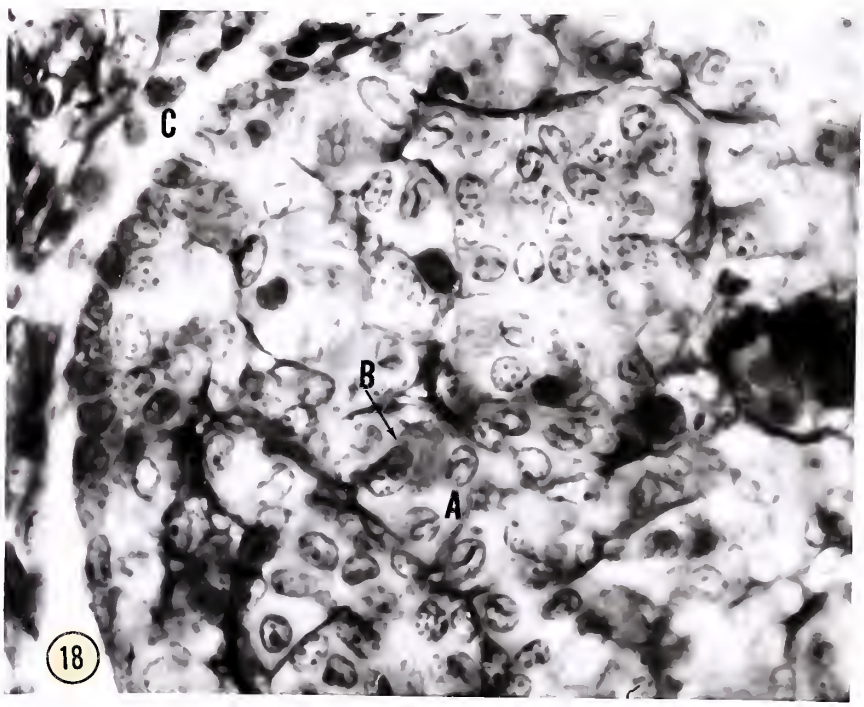
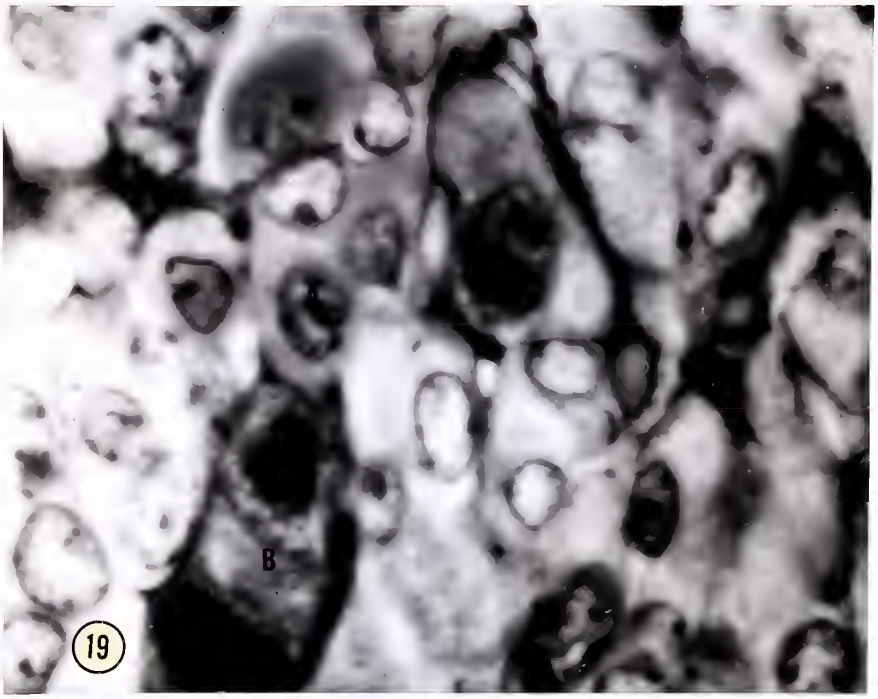

C
B
A
18

FIGURE 19. Same graft as Figure 17 showing acidophiles (A) and a basophile (B). The basophiles have a moderate amount of PAS positive material clumped in the cytoplasm.
2275x

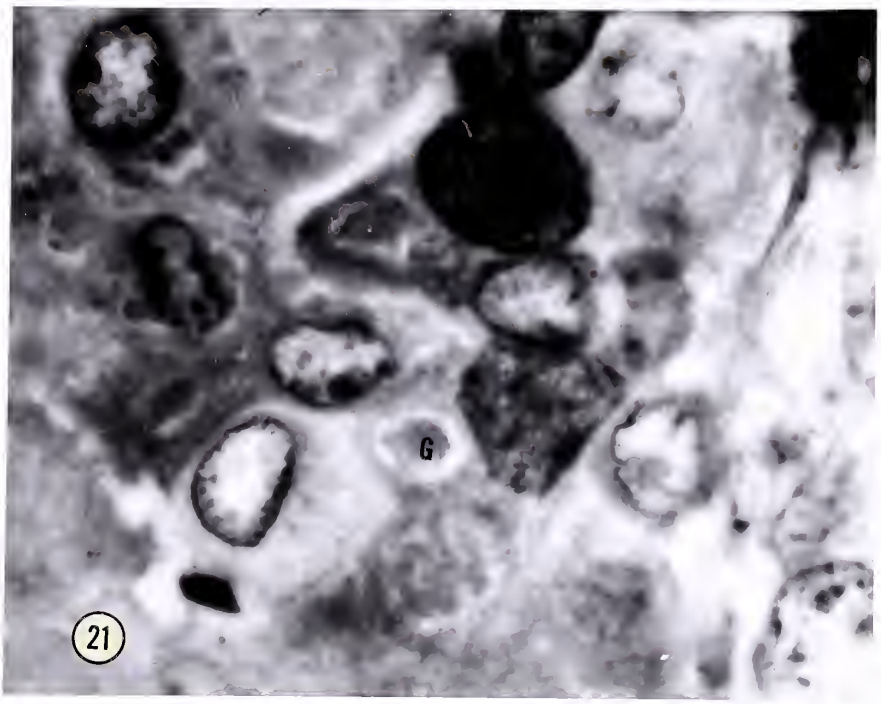
FIGURE 20. Same graft as Figure 17 showing large size of pars intermedia (I) in transplanted pituitary graft. The intermedia cells are arranged in clusters and stain with varying intensity with PAS. PAS-LFB Stain.
235x


B
19


I
20

FIGURE 21. Higher power view of pars intermedia cells from same graft as Figure 17. The cells stain lightly and moderately darkly with PAS and the negative image of the Golgi apparatus (G) is evident in many of these cells. 2275x

FIGURE 22. Section from subcutaneous pituitary graft 14 days after transplantation. Resolution of the necrotic central mass is essentially complete. Pituitary graft cells are arranged in cords along sinusoids. Numerous acidophiles (A) are seen. Moderate numbers of basophiles are identified, but the PAS material is present in sparse coarse granules that stain lightly. PAS-LFB Stain. 880x


G
21

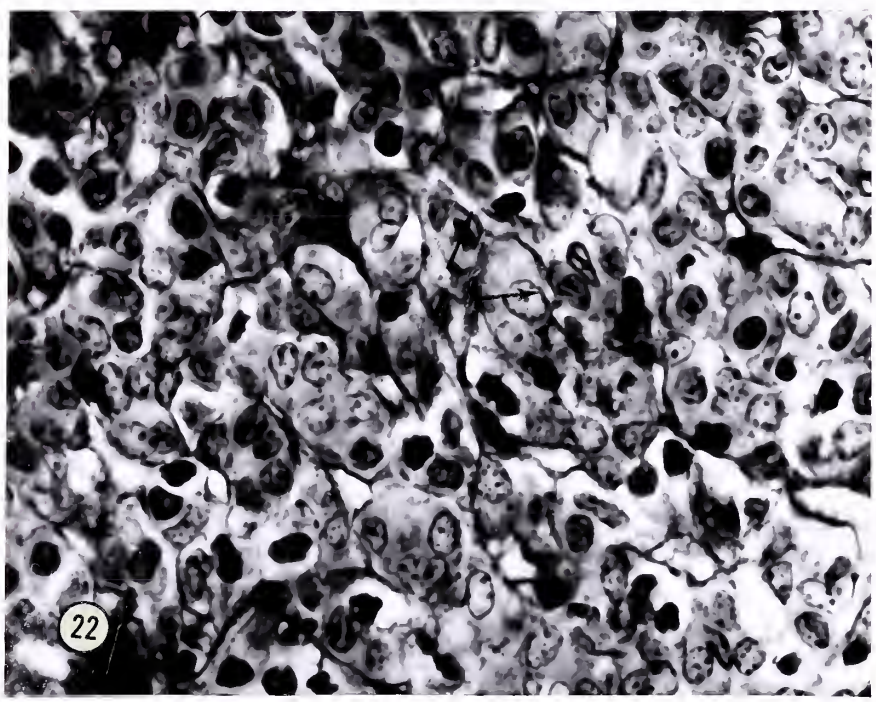
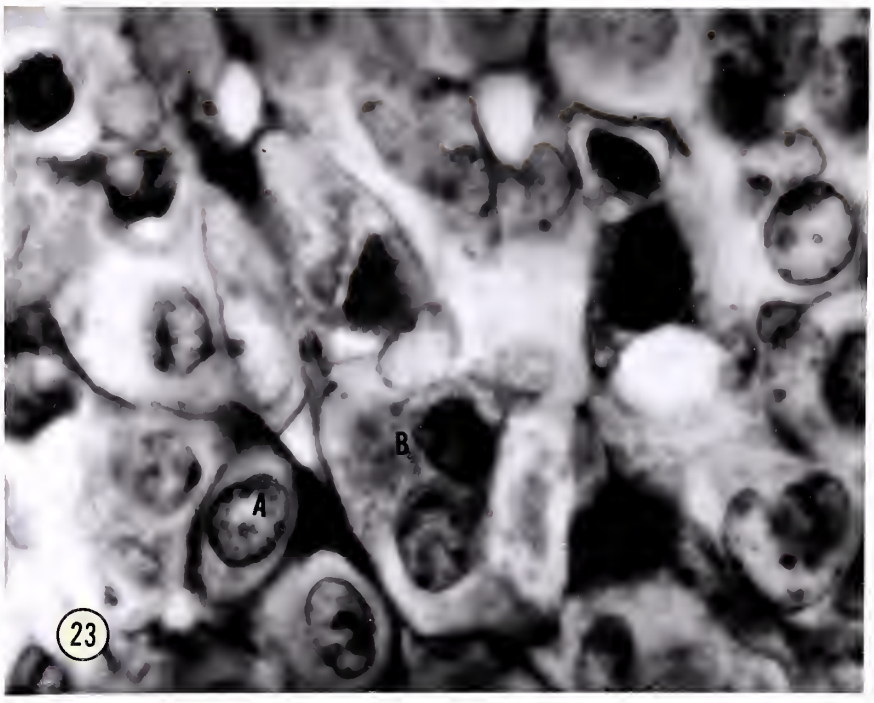

22

FIGURE 23. Same graft as Figure 22 showing the small, intensely stained acidophiles (A) and a lightly stained basophile (B). 2275x

FIGURE 24. Section from same pituitary graft as Figure 22 showing a greatly enlarged pars intermedia with cells arranged in whorls and clusters. 235x


A
B
23

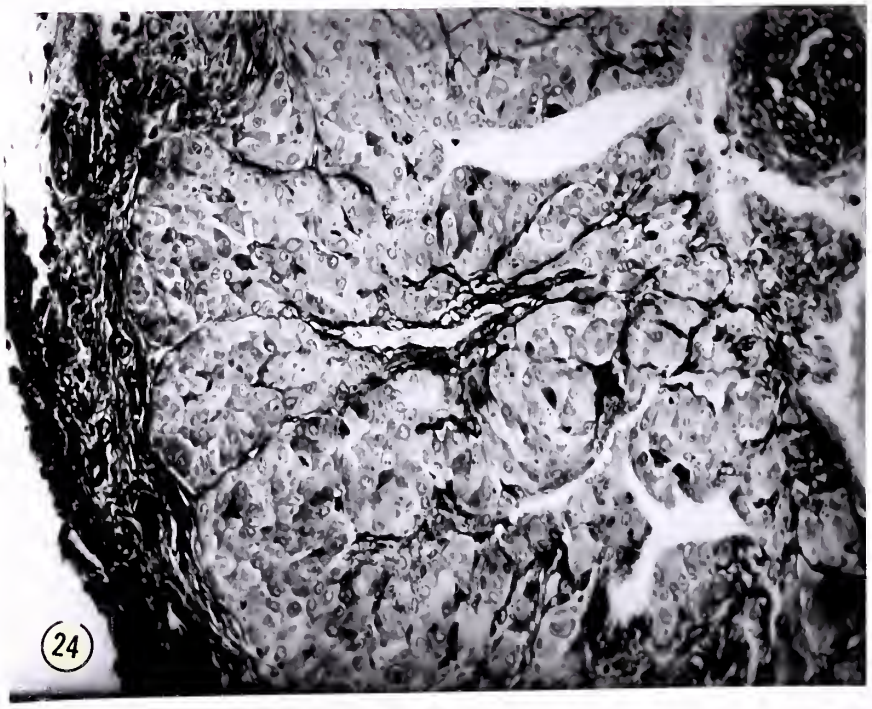
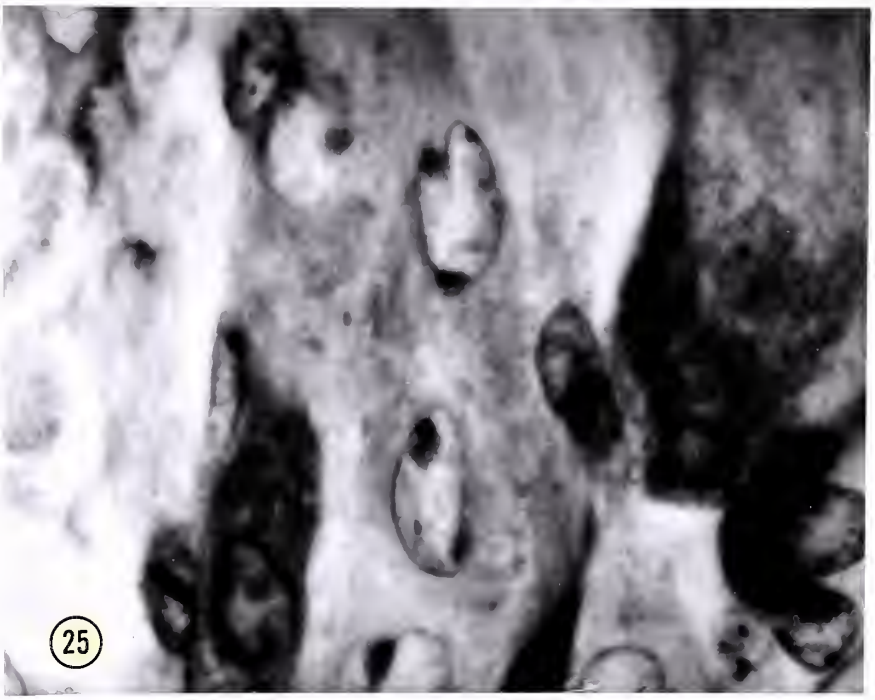

24

FIGURE 25. Enlarged view of Figure 24 showing light and darkly PAS-stained pars intermedia cells. 2275x

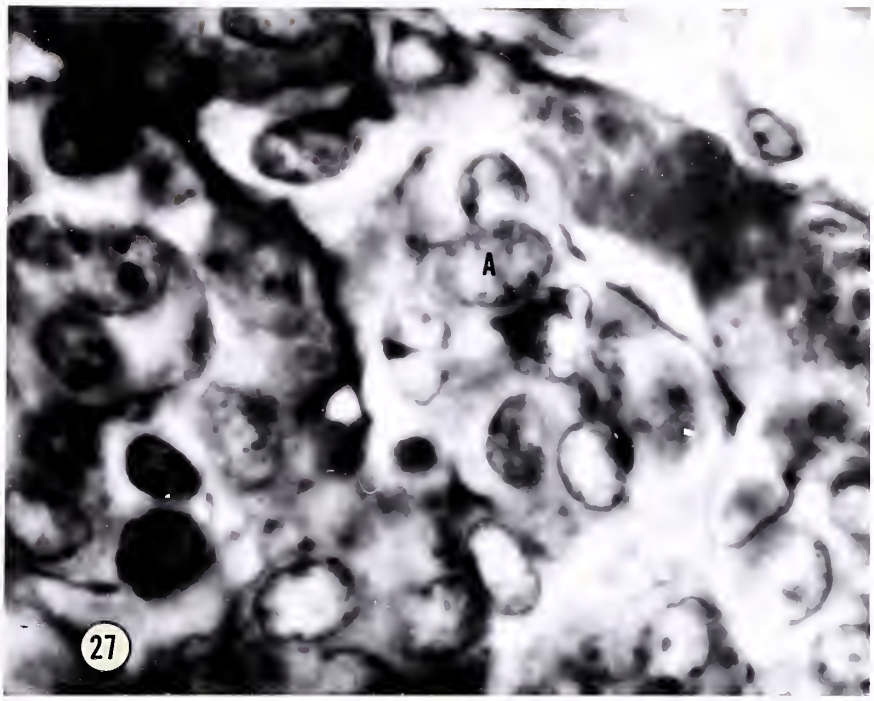
FIGURE 26. Section from subcutaneous pituitary graft 30 days after transplantation. The appearance of the graft is similar to that at 14 days after transplantation, except that basophiles are rare and stain lightly with PAS. Acidophiles (A) are small, oval cells that stain intensely and uniformly with LFB. PAS-LFB Stain. 880x


25


A
26

FIGURE 27. Enlarged view of graft in Figure 26 showing acidophiles (A). 2275x

FIGURE 28. Section from same pituitary graft as Figure 26 showing a greatly enlarged pars intermedia with cells arranged in clusters. Some stain lightly with PAS and others stain more intensely. 235x


A
27

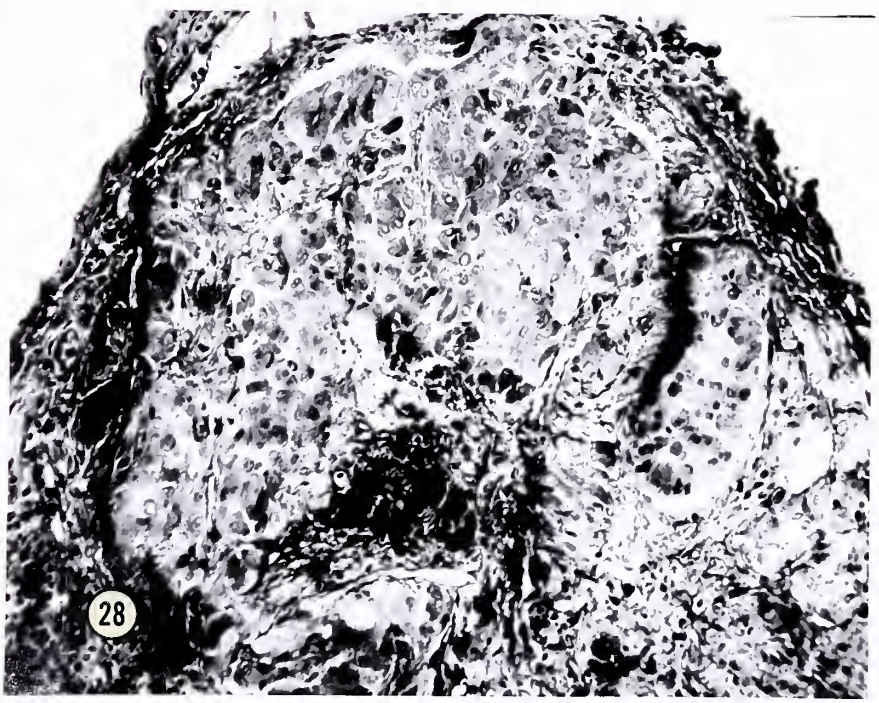
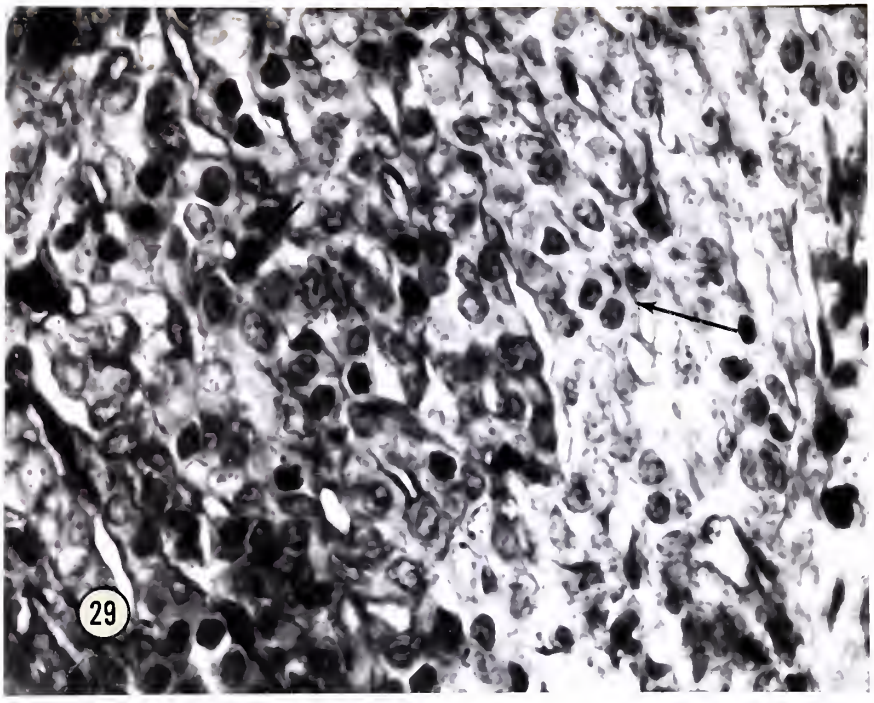

28

FIGURE 29. Section from subcutaneous pituitary graft in a mouse that had been castrated and had received the graft 1 month previously. Numerous acidophiles (arrows) stain intensely with LFB. Basophiles are too lightly stained with PAS to be seen in this photograph. PAS-LFB Stain. 880x

FIGURE 30. Section from subcutaneous pituitary graft in a mouse that had been hypophysectomized and had received the graft 1 month previously. Moderate numbers of acidophiles (arrows) stain with LFB, but basophiles cannot be identified in this photograph. 880x


29

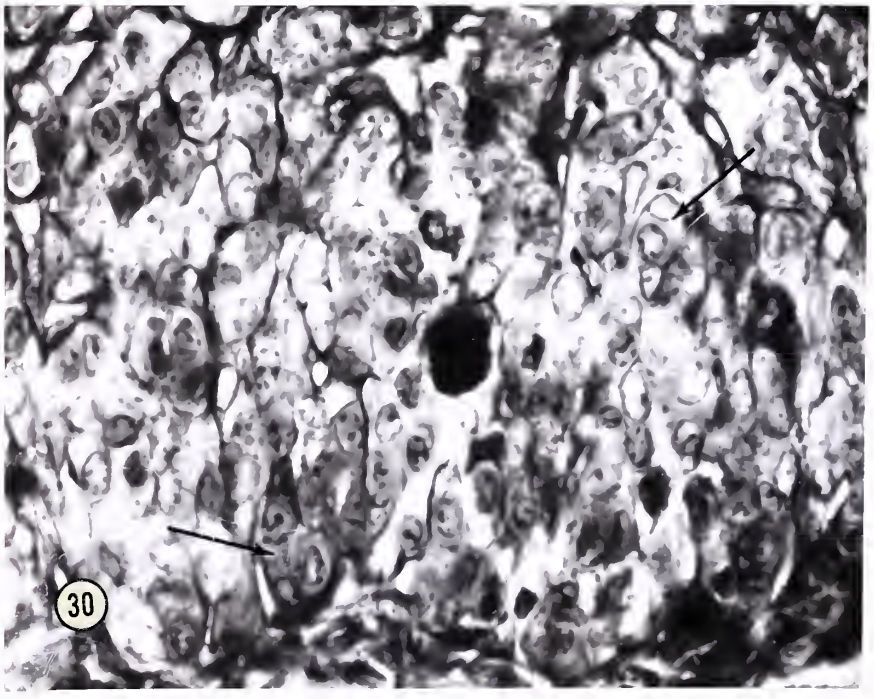
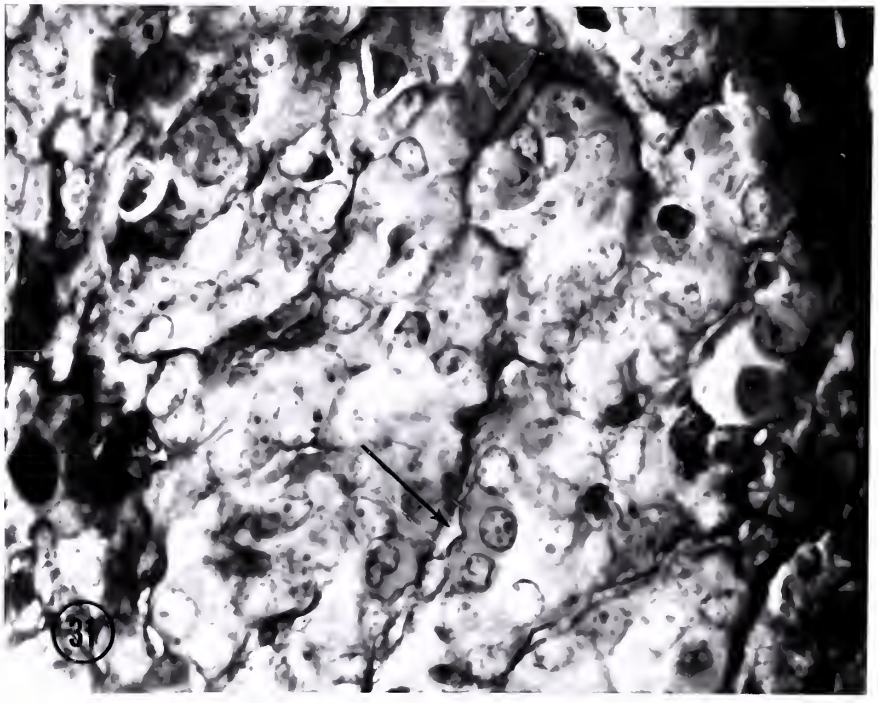

30

FIGURE 31. Section from subcutaneous pituitary graft in a mouse which had been treated with estrogen for 1 month. Acidophiles (arrows) are numerous and somewhat larger than the acidophiles in grafts of intact host animals. Basophiles cannot be identified. PAS-LFB Stain. 880x

FIGURE 32. Section from subcutaneous pituitary graft 60 days after transplantation. The appearance of the graft is similar to that at 30 days after transplantation. Acidophiles (arrows) are small and are moderate in number and staining intensity. Basophiles are not identified. PAS-LFB Stain. 880x


31

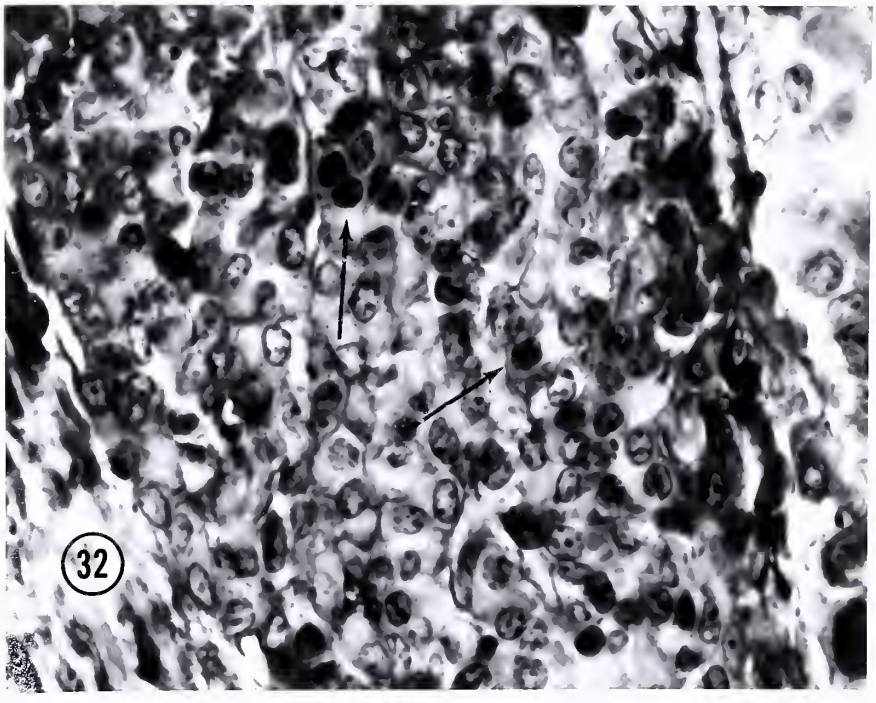
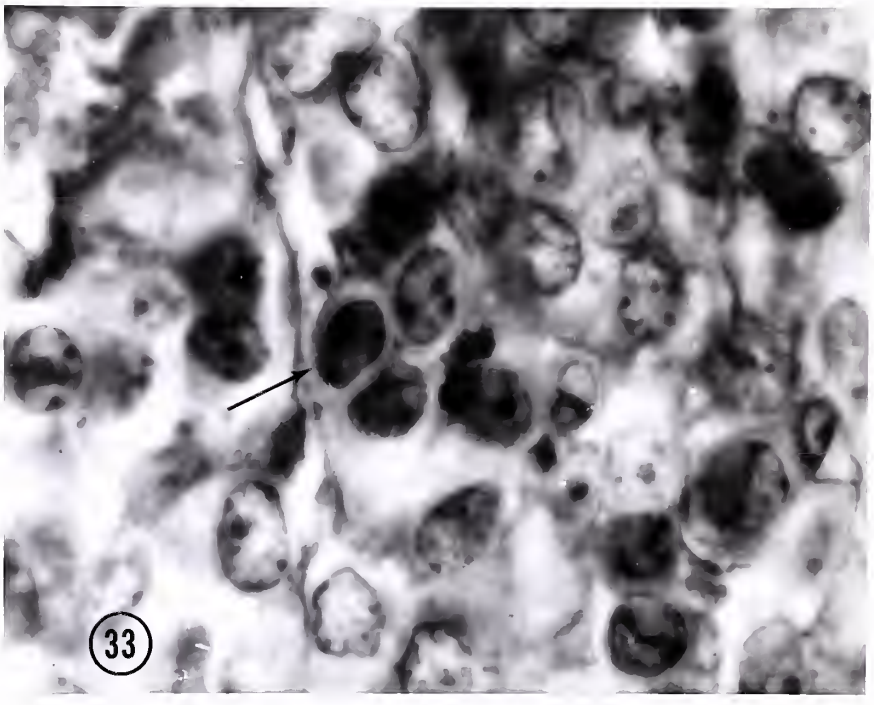

32

FIGURE 33. Enlarged view of graft in Figure 32 showing acidophiles (arrows). 2275x

FIGURE 34. Section from same pituitary graft as Figure 32 showing pars intermedia cells. These cells stain in varying intensity with PAS. 2275x


33

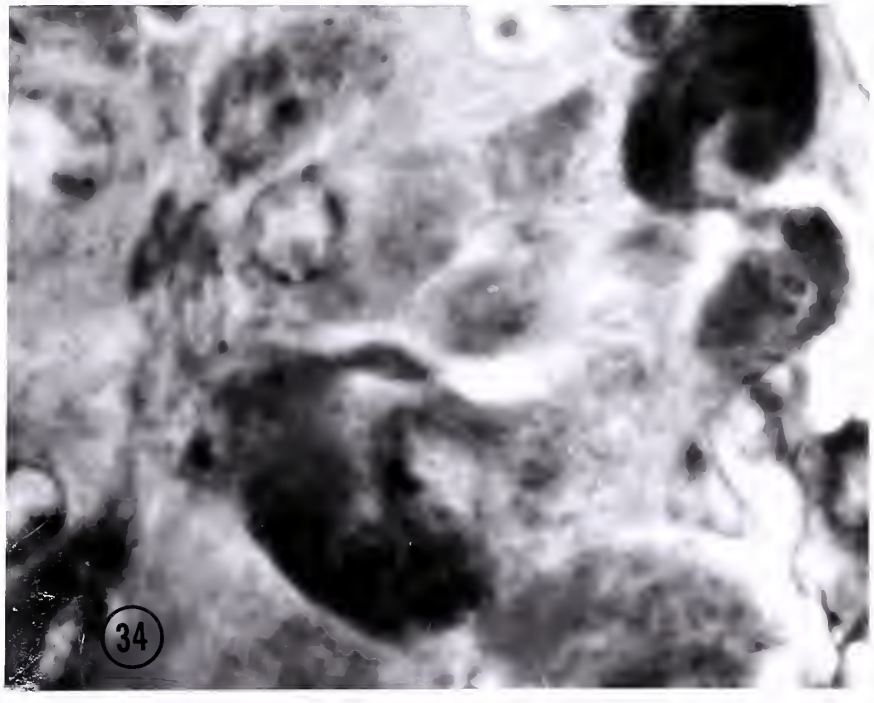
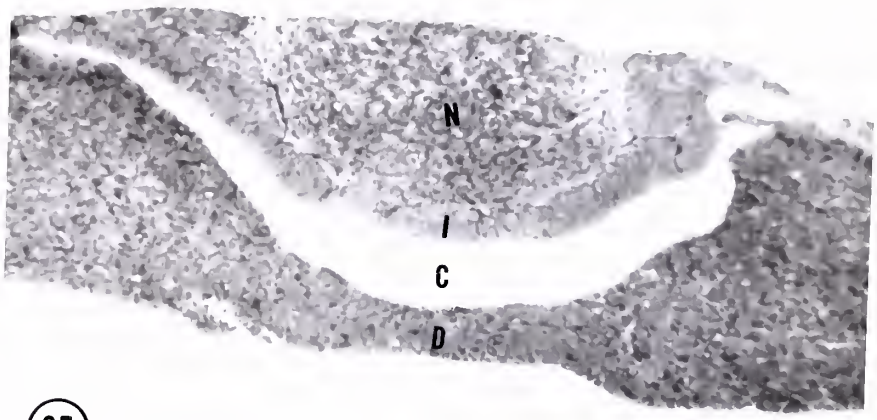
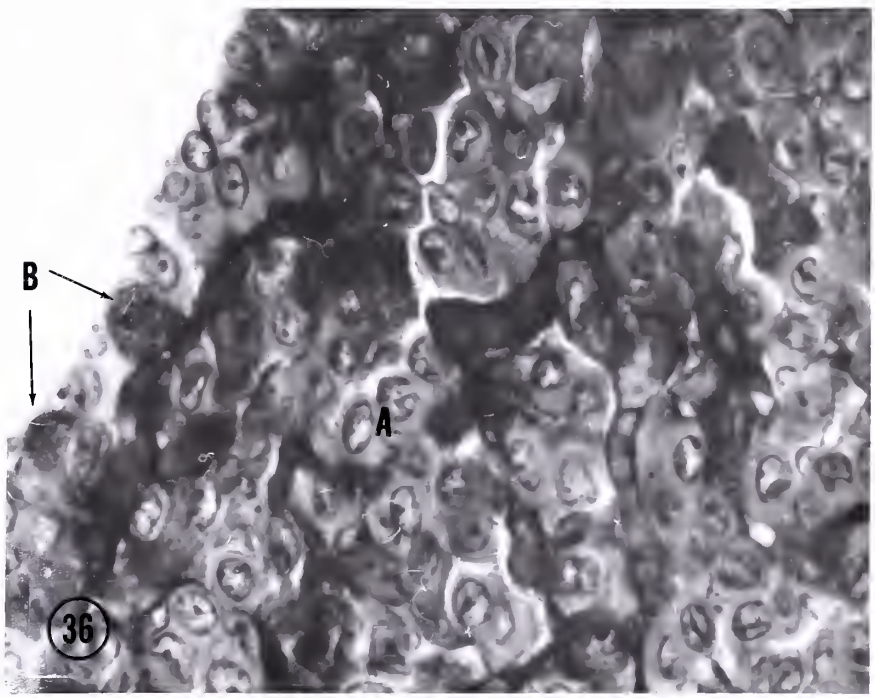

34

FIGURE 35. Frontal section through a normal male pituitary gland stained with PAS-LFB. The neural lobe (N), pars intermedia (I), and pars distalis (D) are indicated. The "sex zone" is the area of the pars distalis immediately below the intraglandular cleft (C) along the narrow isthmus connecting the two lateral lobes. PAS-LFB Stain. 93x

FIGURE 36. Section through the pituitary gland in Figure 35 showing the region of the "sex zone" with many basophiles (B) with intensely-stained PAS material in the cytoplasm. Acidophiles (A) are also numerous and intensely stained with LFB. 880x

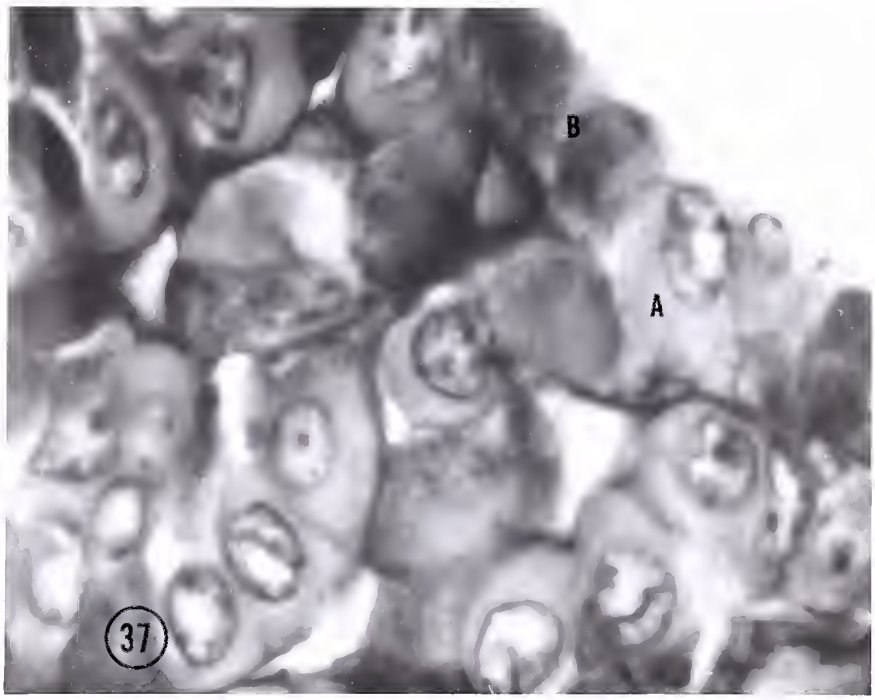


35



36

FIGURE 37. Section through the pituitary gland in Figure 35 seen in higher magnification showing the intensely stained acidophiles (A) and PAS-positive basophiles (B) in the region of the "sex zone." 2275x


B
A
37

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

_____

_____

NAME AND ADDRESS DATE